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STUDIES ON THE BACTERIOLOGY OF THE URINE IN COOPERATION WITH CATHETERIZATION OF THE URETERS*

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THE purpose of this paper is a report of the laboratory studies of urine from cases examined by cystoscopy and ureteral catheterization. The report includes microscopic and bacteriologic results as well as a clinical survey of the cases from the diagnostic standpoint.

The work is based on 162 cases, of which 50 have been reviewed in detail and tabulated in this paper. Frank cases of bladder, ureteral, and kidney involvement make up the majority (35) of the report but there are a large number (15) in which the examinations were made as a part of the routine study. In this latter number are patients giving vague abdominal symptoms which might be referable to most anything in which at least a careful urinary study with negative findings would be valuable from the standpoint of diagnostic elimination.

While I would not advocate the routine use of the cystoscope and ureteral catheter as one might a vaginal examination or the proctoscope, I am sure that its more frequent use where the urine is studied bacteriologically would clear up an otherwise obscure diagnosis, would eliminate the kidney or bladder as offenders, or would accurately clinch a diagnosis strongly suggested clinically.

For purposes of study the cases may be arbitrarily divided into three groups. First, those with clear-cut symptoms in which the laboratory merely confirms as, for example, the findings of streptococcus in a ureteral urine

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where the x-ray has already shown a stone. Second, those in which there are symptoms, for example, pus in the urine, where the laboratory makes a diagnosis of B. coli cystitis. Third, those in which there are vague symptoms and the laboratory findings complete the negative side of the clinical examination, thus eliminating the urinary system. In this study of 50 cases, 21 are in Group I, 14 in Group II and 15 in Group III.

Complete cooperation with the clinical service is necessary so that observations may be accurately recorded and different phases compared. Specimens must be sent to the laboratory with the least possible chance of contamination, clearly and correctly labelled. It has been my pleasure to work with J. P. Shearer and E. H. Adams, most of the cases with the latter and I am indebted to them for the best kind of cooperation.

The methods employed have been as simple as possible with a routine which has varied very slightly since the work was first undertaken. The patients receive a preliminary routine urine examination and often the presence of pus cells is the first indicator of a serious trouble. In this routine the presence of albumin, casts, pus cells, white blood cells and blood cells with types of epithelium are considered the most important elements to be watched for. Amounts indicated by 1, 2, 3, 4, are recorded for positive findings. While other things such as crystals, debris, urates, and bacteria are set down they are considered of secondary importance.

When the patient is put on the list for a cystoscopic examination, special specimens of urine are obtained under conditions as nearly aseptic as is surgically possible. About 10 c.c. in a test tube is sufficient except where tubercle bacilli are suspected when it is better to collect five or six test tubes from each ureter.

The specimens are given a number and are examined,—bladder, right ureter, and left ureter in order. The amount, color, sediment, precipitate, gross blood, and pus are noted. The specimens are centrifuged at high speed and the sediment used for all further examinations. Fresh smears are examined under dry powers with reference to white blood cells, pus cells, red blood cells, types of epithelium, and bacteria. Gram stained slides are next examined with the oil immersion for the presence and types of bacteria. Smears are fixed on slides on a copper plate, stained for tubercle bacilli by the cold method, which I advocated several years ago, and examined for at least five minutes and much longer where tuberculosis is suspected or where the tubercle bacillus has been found previously in a twenty-four-hour specimen. Cultures are made in a gas tube of glucose broth and by smear on blood agar plates. The cultures may be made the first thing before centrifuging or by loop transfer afterwards. Gas tubes, when positive, are transplanted to Endo's medium for B. coli and when there is a cloud and no gas, they are transplanted to blood agar plates. It is rarely necessary to fish colonies to separate plates since almost every case shows but one infecting organism. There is no flora in the bladder under perfectly normal conditions and the urine is probably sterile most of the time.

This technic is applied to each of the three specimens in order. Where an organism out of the ordinary is found, special media and methods are

used to work out special characteristics. In this series which I realize is very small, I have been dealing with a surprisingly small number of different species. Where a vaccine is in order, salt solution suspensions of scraped blood agar plates are made from the original cultures, if pure and heavy enough, or if not, from the first transplants available.

There are 162 cases in the series at this time and 50 have been carefully reviewed.

These include cystitis, 6; cystitis with pyeloureteritis and urethritis, 8; pyeloureteritis, 9; pyeloureteritis with stricture or pregnancy or renal calculi, 7; hydronephrosis, 1; pyonephrosis, 2; nephrolithiasis, 3; tuberculosis of the kidney, 3; tumor of the kidney, 1; and ptosis of kidney, 1. The negative cases include neurasthenia and neurosis (observation negative), chronic appendicitis, myocarditis and gall bladder, perineal tear and pelvic symptoms, gonorrheal prostatitis, purpura, chronic salpingitis, tuberculous epididymitis and Bartholin's abscess.

There were 12 males and 38 females. These types of cases are by far more frequent in the females. The youngest was five, the oldest sixty-four, average thirty-four years; the largest number were between the ages of twenty-five and thirty and again between forty and fifty, thus these diseases belong to middle adult life. The longest duration of symptoms was five years, the shortest one week, the average fifty weeks, and the greatest number between one and six months; the acute cases were between two weeks and two months and the chronic cases between one and two years.

In a study of the symptoms produced there is a wide variety, but pain of some character and position occurred in every case. Frequency was present 36 times, burning during and at the end of micturition 21 times, hematuria 18 times, nocturia 13 times. Then follows about equally distributed a long list of symptoms which were noted, such as backache, headache, weakness, chills and fever, tenderness, dribbling, etc. Involvement of some part of the urinary tract is characterized by pain, frequency, burning, hematuria and nocturia in order of frequency in this series.

Nineteen cases showed frequent and intermittent attacks before coming to the hospital, emphasizing the passage in cycles of these forms of urinary diseases.

In the preliminary urine report albumin was present in forty-three specimens from a trace to 4, mostly the former. This was due to blood or pus and not to an organic nephritis. Blood cells show up 21 times and pus 28, an exudative reaction proving the infectious nature of many cases in this class. Tubercle bacilli were demonstrable in twenty-four-hour specimens 3 times and were not found twice where specifically looked for.

In the cystoscopic specimens taken from the bladder fresh smear examination, pus cells appeared 26 times, white blood cells, 3; epithelium, 15; red blood cells, 18; bacteria, 6. Gram stain,—bacteria, 19; tubercle bacilli were never found in these specimens. Cultures,—tubes sterile, 9; gas positive, 20; cloud and no gas, 10; Endo positive, 12; blood agar plates, sterile, 17, growth, 23. B. coli occurred 17 times, Streptococcus hemolyticus, 6; Staphylococcus

albus, 5, hemolytic staphylococcus, 1; B. fecalis alcaligenes, 1; Streptococcus pyogenes, 1; undetermined B. coli Group II.

In the cystoscopic specimens taken from the right ureter fresh smear examinations, pus cells appeared 20 times, white blood cells, 6; epithelium, 22; red blood cells, 18; bacteria, 1. Gram stain,—bacteria, 11; tubercle bacilli were found once. Cultures,—gas tubes sterile, 22; gas positive, 8; cloud and no gas, 8; Endo positive, 5; blood agar plates, sterile, 24, growth, 14. B. coli occurred 8 times, Streptococcus hemolyticus, 1; Staphylococcus albus, 6; hemolytic staphylococcus, 1; Streptococcus pyogenes, 1; B. fecalis alcaligenes, 1.

In the cystoscopic specimens taken from the left ureter fresh smear examination, pus cells appeared 13 times; white blood cells, 7; epithelium, 19; red blood cells, 19; bacteria, 1. Gram stain,—bacteria, 3; tubercle bacilli were found once. Cultures,—gas tubes sterile, 26; gas positive, 3; cloud and no gas, 7; Endo positive, 3; blood agar plates, sterile, 27, growth, 8. B. coli occurred 5 times, streptococcus hemolyticus, 1; hemolytic staphylococcus, 1; Staphylococcus albus, 3; B. fecalis alcaligenes, 1.

The same culture was present in the bladder and both ureters 8 times, in the bladder alone, 13; bladder and right ureter, 9; bladder and left ureter, 1; left ureter alone, 2, and not once in the right ureter. Infection appears most commonly in the bladder alone, next the bladder and both ureters, and is more common on the right side than on the left. The ureters are rarely involved without bladder infection.

The cystoscopic examination was negative in 14 cases, showed evidence of cystitis in 9, involvement of the trigone in 9, obstruction of the ureters in 6, and such other findings as granulation, swelling and thickening of the mucosa, swelling of ureteral orifices, trabeculations, ribbon bands of exudate, mucosal folds, contractions, slough, and the presence of tumor.

The autogenous vaccine was prepared in 16 cases. The organisms used included B. coli, Streptococcus hemolyticus and Staphylococcus albus. They were made from the original cultures as nearly as possible, put up in saline suspension so that 1 c.c. equals approximately five hundred million organisms, killed at 60° C. for one hour and given in ascending doses starting with 0.1 c.c., increasing 0.1 c.c. every other day and continuing for several weeks in 1 c.c. doses. In the stubborn cases where they were of benefit, they produced complete results and, while this paper does not deal with treatment, a vaccine should be tried in every case where other measures cannot be used. One typical case might be cited illustrating this point. A woman, aged twenty-eight, began with frequency, urgency, abdominal pain and pus in the urine of one year's duration. Cystoscope showed distinct evidences of cystitis and the Staphylococcus albus was isolated from the bladder and both ureters. The patient was kept in bed for four weeks with local treatment of boric acid and mercurochrome irrigations. This treatment was supplemented by a vaccine which was faithfully carried out during a further period of five weeks of semirest. Control cultures were made on the bladder which finally became sterile. The patient's symptoms completely subsided; she gained

twenty-five pounds in weight, and has enjoyed perfect health for the past year.

There was a bad dental infection present in 5 cases. This number would undoubtedly have been increased if the teeth of these patients had been examined routinely.

Vaginal smears were positive for gonococcus but once, and a prostatic culture was positive once. The gonococcus plays a small part, if any, in bladder and kidney infections.

Blood serologic tests were made on 45 of the 50 cases; 4 were positive and 41 negative. This percentage is slightly higher than our routine hospital cases, but syphilis seems to have played no direct part as an etiologic factor.

There were 6 operations in the series, 4 nephrectomies, 1 nephrolithotomy, and 1 perineal repair. Except for stone and tuberculosis, the less surgery the better. This series of 50 does not include surgical kidney which is among the larger group.

There are many facts of interest gleaned from these cases which might be profitably included in this paper and set down in a more or less verbatim fashion.

Where B. coli is present in large quantities in the bladder, they may appear in cultures from the ureters although the latter are not involved. This could be determined by the presence or absence of pus cells and bacteria in fresh and Gram smears.

The large number of negative cultures with negative cystoscopic findings and vague symptoms act as a check on the other work reported in this paper.

In a case of pyonephrosis on one side, examination of the corresponding ureteral urine showed marked pus and cultures while the opposite side was perfectly clear. Subsequent nephrectomy proved an infected kidney, and complete recovery proved the other kidney to be normal.

Cases of stone in the ureter may show the presence of much pus from the affected side with sterile cultures.

A case of severe perineal laceration with vague bladder symptoms and B. coli cultures positive cleared up following a repair and no treatment directed at the bladder.

Hematuria is not only a sign of tumor, stone, or tuberculosis, but is present to an alarming degree in hemolytic streptococcus infections. The red blood cells under these conditions are likely to show hemolysis with hemoglobinuria.

Stone in the pelvis of the kidney may be present without any symptoms as in one case where albumin was found at an insurance examination. Subsequent careful study revealed the albumin due to pus, the pus coming from one ureter, and the x-ray revealed the stone.

The gonococcus plays a small part in the series, but it was isolated from the bladder in one case. This was probably more a prostatitis with urine contamination than a real etiologic factor in the bladder.

Where the tubercle bacillus is demonstrated in a twenty-four-hour spec-

imen followed by its presence in one ureter and not in the other, this should be confirmed by repeated examinations before a nephrectomy is advised, because of the frequency in which both kidneys are involved in nephritic tuberculosis.

Several cases with coincident dental infection constitute another link in the study of focal infection and, where cocci are found, teeth and tonsils should always be investigated.

In tuberculosis of one kidney, where pus is pouring out of the infected side with the demonstration of tubercle bacilli without difficulty, and where the mouth of the ureter is granular while the opposite side has clear urine and no ureteral involvement, a diagnosis of unilateral tuberculosis is justified and nephrectomy may be recommended.

Cases of ureteral obstruction following pelvic surgery will show a marked increase in the amount of ureteral epiderm with sterile cultures.

Where urinary system symptoms are present and the case cannot be completely studied for one reason or another, it is impossible to arrive at a diagnosis and unless this case be completed, it had better be dropped.

A case of Streptococcic hemolyticus cystitis failed to clear up under local treatment and vaccine until a pair of bad tonsils were removed and organisms isolated from this source added to the vaccine.

In a case of adenocarcinoma of the kidney, the urine from the ureter on the affected side showed a large number of large unusual cells other than ureteral undergoing necrosis. Such a finding might be considered a suggestive diagnostic point.

A case of ptosis of the kidney, the diagnosis being made by physical signs and the x-ray, gave negative cystoscopic and bacteriologic findings.

In commenting on the high lights of this study, I should say first of all, that complete cooperation between the clinical side and the laboratory must exist with perfect coordination. The material must be received as nearly the same as it is present in the body as possible, and examination must be made immediately.

A simple method of procedure must be worked out in the laboratory. This should include a means of detecting all possible changes which might throw some light on the diagnosis.

The method should differentiate between the bladder and the ureters and between each ureter. It should include a study of the fresh smears, the stained smears (preferably by the Gram method), tubercle bacilli staining, and some forms of routine culture media which will bring out especially B. coli and the cocci groups.

A tentative diagnosis may be made from the bacteriologic studies by the pathologist and, if not a definite diagnosis, at least an interpretation of the findings should be set down. Where this is followed by a conference with the clinician, valuable suggestions come from both sides.

Finally every case is a study in itself and while they fall into general groups and types, many varied pathologic lesions have much in common as to symptomatology and must be completely worked out by the clinician, the

cystoscope, the x-ray and the laboratory. No one group is sufficient unto itself.

DISCUSSION

Dr. A. H. Sanford.—There is one thing I would like to ask Dr. Keilty as to whether he doesn't use the guinea pig inoculation for diagnosis. We find that the inoculation of guinea pigs with urine from each kidney has always been a great aid to the surgeon. We routinely divide the specimen of urine and inject part of it subcutaneously and part of it intraperitoneally. I do not know whether there is any reason for it, but sometimes one is positive before the other one. I would also like to ask what stain he used.

Dr. A. I. Rubenstone.—It is a good thing to bear in mind that tuberculosis can be diagnosed in the course of routine urine examinations, especially where many urines are examined. If technicians bear in mind their rule to set aside all pus urines that are acid and of low specific gravity, it is surprising how often one can pick out tubercle bacilli from these specimens when, especially in hospital practice, it might be overlooked. Differential staining by Pappenheim's method has been a great aid in this work. One patient particularly is interesting. A prominent man in the community was told that he had tuberculosis of the urinary tract because acid-fast organisms were found in his purulent urine. When the urine was examined with the ordinary Gabbet's stain, red bacilli were seen in some number but with Pappenheim's rosolic acid stain none could be demonstrated. Further investigation of the patient and guinea pig inoculation proved entirely negative for tuberculosis.

Dr. Frank W. Hartman.—We have found that if a method of concentration is used on suspected tuberculous specimens the bacilli are present in sufficient numbers to be demonstrated by almost any of the methods of staining mentioned. The concentration method of Andrus & MacMahan is quite satisfactory.

Dr. H. J. Corper.—I do not wish to talk about staining tubercle bacilli now as this will be dealt with fully in my paper. The subject of examining for tubercle bacilli in the urine is an important one. I personally am not willing to make a definite diagnosis of tuberculosis from the urine unless the microscopic findings are verified by guinea pig inoculation findings, since there are so many possibilities for error in the usual methods. For that reason I prefer to wait in every case before making a definite diagnosis, unless all other evidence points to tuberculosis and the time factor is paramount. It is surprising that many physicians depend implicitly on the finding of a few bacilli in the urine without considering the other pathologic findings in the case or the chances for error in the microscopic method with urine. A negative microscopic finding likewise is of very little import because so little material can actually be examined by this method. Our only safeguard to avoid error and make the diagnosis more certain is to inject a guinea pig in which 10 to 100 bacilli can produce tuberculosis, and by means of which all other acid-fast forms, except human and bovine tubercle bacilli, are excluded because they do not produce the characteristic generalized tuberculosis in this animal in from one to three months.

Dr. A. H. Schade.—The guinea pig does not always die; sometimes it is necessary to kill the pig. Many survive and have extensive tuberculosis.

Dr. John A. Kolmer.—I may endorse the statements of the last speaker and believe it always advisable to perform a necropsy on all guinea pigs even when inoculated by subcutaneous injection, since tuberculosis of the internal organs and especially of the spleen, when the external lymphatic glands have apparently escaped infection.

I wish to ask Dr. Keilty his opinion of the possibility of excretion of tubercle bacilli in the urine of individuals without tuberculous infection of the genitourinary organs.

Dr. A. S. Giordano.—About a month ago I had an opportunity to do a postmortem on a patient about sixty-five years old with acute miliary tuberculosis and no pus found in the urine before death. We found tuberculosis of the lungs, spleen, and liver. The kidneys were covered with little tubercles; there was no pus found in the urine at this time nor any tubercle bacilli in the smears, while the pig inoculation was positive.

Dr. Robert A. Keilty (closing).—I did not mention guinea pigs in my paper. I believe that one may tell the tubercle bacilli from experience; I believe that one may find microscopically the tubercle bacilli if one is sure of the stain, etc., and that one may make a diagnosis by the findings under the microscope. I do not see that the guinea pig is important or essential. We all have our own ideas about staining. I believe that rosolic acid is a very good stain. I also use a stain of my own. I do not believe that the tubercle bacilli are in the circulating blood in any great numbers. The possibility of the excretion of tubercle bacilli in the urine is not probably very great although it is to be thought of.

STUDIES IN EMBALMING FLUIDS IN RELATION TO NECROPSIES*

By John A. Kolmer, M.D., and Fred Boerner, V.M.D., Philadelphia, Pa.

WITHOUT entering into a general discussion of the reasons why permission for necropsies is so frequently denied or withdrawn in hospital and private practice, it may be stated that one of them is the objections raised by undertakers. The whole subject has been recently investigated in a most excellent manner by a committee of the Pennsylvania State Medical Society, composed of Dr. Frank C. Hammond (chairman), Dr. V. L. Andrews and Dr. David W. Thomas, who made a survey of the attitude taken by undertakers in opposing the obtaining of consent for postmortem examinations, and in their report[1] it is stated that "the undertaker too frequently urges the family not to give permission, or, if permission has been given, to withdraw it. His reasons for doing so are that: it is impossible properly to embalm a body that has had a postmortem done; it increases the cost of his services; the body will not be turned over to him in 'decent condition'; and, it will not be possible to make the remains presentable. If the proper technic is observed in making the postmortem, no difficulty should be experienced in embalming. The medical profession is to blame in many instances for carelessness in technic and we must correct our indifference if we are to secure the cooperation of the undertaker. If a proper technic is followed, there should be no excuse for the undertaker objecting to an autopsy or making an additional charge for his services. The better class of undertakers will not do so. When a family finds that the undertaker has charged ten to twenty-five dollars extra on account of the postmortem, it will have its baneful influence."

In the hospitals of the Graduate School of Medicine of the University of Pennsylvania we have frequently been denied permission for necropsies on account of this attitude on the part of undertakers, particularly in the case of the burial of individuals of reduced financial status requiring funerals of the minimum expense. Undoubtedly the embalming of a necropsied body requires more time and skill on the part of the undertaker, although as stated by the committee referred to above, the well-trained and experienced morti-

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cian seldom if ever raises any objections and indeed, in our experience, has usually given the most friendly and helpful cooperation. On the other hand it cannot be denied that some morticians will slyly raise objections to the necropsy with the friends or relatives and block permission or even have it withdrawn, despite the assurances of the pathologist to exercise all the usual technical precautions in conducting the necropsy in order to facilitate successful and satisfactory embalming. Among the more important of these technical precautions may be mentioned: (1) To make the routine incision from below the suprasternal notch to the symphysis pubis, except in women, when the incision should not be as high or of the Y-shaped kind, in order to permit the wearing of the customary low neck shroud; (2) to exercise every precaution against undue disfigurement if other incisions are required and to avoid carefully all unnecessary incisions; (3) to keep the circulatory system as intact as possible by ligating all vessels before removing any of the viscera of the chest or abdomen; (4) to examine the aorta *in situ,* but if removed, to ligate the left carotid and innominate arteries, leaving eight inches of ligature attached; in other words to tie off all large arteries and veins severed in doing the work after the blood has drained out. (The vessels tied are the following: The right innominate, the left carotid, and the left subclavian, and the two innominate veins in the neck; the renal arteries, the celiac axis and the superior and inferior mesenterics. In case the aorta is removed to tie both femoral arteries and veins.) (5) To ligate the rectum below the line of incision if the intestines are removed; (6) to remove the brain by a scalp incision from ear to ear over the vertex and to prevent leakage from the carotid arteries by plugging, or tying off at the base of the skull, with plaster of Paris or other means; (7) to keep the head and shoulders raised during the examination in order to allow the blood to drain away; (8) if the body is turned face downwards for removing the spinal cord, to protect the face and especially the nose, against bruising; (9) to sponge out all blood from the cavities and pack adequately, and (10) to sew up tightly and neatly and remove all blood stains. In addition to these precautions of considerable importance to the mortician, all of them reasonable and readily complied with, the pathologist should show him the courtesy of delivering a clean body as nearly as possible at a specified time. Sometimes it is considered advisable to permit the undertaker to embalm the body before the incision is closed and especially in cases where extensive removal of viscera has been done.

In small communities where the pathologist and morticians may be personally acquainted and an understanding on these points reached by working together and in harmony, difficulties are not likely to arise, but in a large city it may be otherwise, and the fact remains that not a few of the so-called little undertakers lose no time in obtaining immediate possession of the body in order "not to lose the job," and in Philadelphia at least some will not hesitate to advise the friends and relatives against permission for a necropsy or to have it withdrawn in order to get the "job" away as quickly as possible and to minimize the amount of work and skill required for preserv-

ing the body for the funeral. Furthermore, all undertakers are anxious to embalm as soon after death as possible in the interests of satisfactory preservation of the body.

Such being the circumstances, it has occurred to us and doubtless to many other pathologists who have lost necropsies on account of this attitude on the part of some undertakers (but probably sometimes justified by carelessness and lack of courtesy on the part of the pathologist) to give more attention to the possibility of inviting the undertaker to embalm first and to provide him with an embalming fluid if necessary along with adequate working facilities. By reversing the usual order in this manner we may at least remove his objections even though it is to be realized that they have but little real justification at best, providing the pathologist is willing and capable of observing the above-mentioned precautions.

One insurmountable objection to this plan, however, is that bacteriologic cultures cannot be made after embalming; chemical analyses of organs or the gastrointestinal contents may likewise be disturbed or rendered entirely useless, but the latter are required in but a very small percentage of cases and in reference to the former, means may be made when bacteriologic examinations are required. In so far as cultures are concerned, embalming fluids invariably possess a high degree of bactericidal activity to fulfill their functions, rapidly sterilizing the blood and fixed tissues with which they come in contact.

As all pathologists of experience are aware, a fairly satisfactory necropsy can usually be made on an embalmed body in so far as the detection of the gross pathologic lesions are concerned. It is true that the organs and tissues may be rendered tough, leathery and blanched, by the formalin incorporated in almost all embalming fluids, or discolored by red dyes, which may render difficult or impossible the macroscopic detection of the finer tissue changes, but the detection of the gross changes is usually quite possible, most difficulty being experienced in the detection of embolism and thrombosis, since the usual embalming fluids quickly convert residual blood into tough gummy masses.

As we have observed in numerous experiments with various embalming fluids to be found in the market, the histologic examination of embalmed tissues is oftentimes difficult, especially from the standpoint of vascular changes, since the undertaker does not remove all blood from the internal organs by his usual technic and the sections almost invariably show extensive congestion and some interstitial hemorrhage.

From the standpoint of the mortician an embalming fluid must serve the following fundamental purposes: (1) Preserve the body against putrefactive changes for at least the usual period; (2) fix and harden the tissues and at least the muscles of the face to prevent dropping of the jaws when the rigor has passed away, and (3) as far as possible, replace the pallor of death with some more lifelike color, especially the skin of the exposed parts. To secure these ends he must inject sufficient fluid for the preservation of the skin, and it is imperative that the fluid injected shall be sufficient for preserving the

body after the necropsy has been done, although it is readily possible for the mortician to reenforce his efforts by injecting additional amounts of embalming fluid into the thoracic and abdominal cavities, subcutaneously or even into the great vessels of the neck or any particular arm or leg, after a complete necropsy has been conducted.

From the standpoint of the pathologist the ideal embalming fluid for injection before necropsy should possess the following fundamental properties: (1) It should not discolor, harden or otherwise alter the physical state of the organs and tissues and (2) it should not alter the residual blood. To these may be added a third property, it should not contain the usual poisons like arsenic, in case chemical analyses are required in medicolegal cases. One is tempted to add that it should not be bactericidal in order that bacteriologic examinations of the blood and internal organs may be made during the necropsy, but this is superfluous when it is remembered that preservation demands that the embalming fluid shall be parasiticidal or at least parasitostatic in action.

During the past two years we have experimented with a number of embalming fluids in common use among undertakers and found all of them to contain so much formalin that when used in the dilutions and amounts recommended, the internal organs were changed in color and consistency with marked changes in the microscopic pictures involving mainly the residual blood. Indeed, some of these fluids contain so much formalin that it is impossible to distribute it evenly through the capillary tree, by reason of producing vascular occlusions, and one cannot escape the conclusion that some morticians depend largely upon the principle of getting sufficient into the great vessels and abdominal cavity to take care of the balance of the body for a few days at least by gaseous distribution of formaldehyde. In discussing this subject Robertson[2] of the Mayo Clinic, possessing a very extensive necropsy experience, states that if the undertaker will use his solution in one-half the usual strength, he will wash out blood more easily, obtain a more intensive penetration of the capillary tree and that he will be more pleased with the results than he would be with his stronger solution, although if the cheeks are not fixed tightly enough he may find it necessary to inject the head with the usual strength solution. Our experiments have amply confirmed Robertson's statements and it is advised that when embalming is done before the necropsy is conducted, but the undertaker be requested to dilute his usual fluid one-half more for injection into the body below the head but that for the head he use the usual strength solution.

But from the standpoint of the pathologist the conditions are not yet just right and the mortician is sometimes required subsequently to take care of patches of skin showing discoloration before burial. From the standpoint of preservation we have had much better success with an embalming fluid prepared by Dr. Addinel Hewson,[3] Professor of Anatomy in the Graduate School of Medicine in the University of Pennsylvania. This fluid contains only one and one-half per cent formalin and therefore permeates the capillary tree very readily and easily, does not cause marked changes in the

physical state of the organs and alters but slightly or not at all the usual histologic appearances. Furthermore, it has been a very satisfactory preservative since we have kept necropsied dogs at a temperature of about 24 degrees C. for weeks and indeed, for months, without putrefaction. Unfortunately, however, it does not harden the muscles of the jaws sufficiently and the low formalin content would make it illegal for embalming purposes in some states.

At this stage of our investigations we were invited by Dr. Hammond, chairman of the aforementioned committee of the Pennsylvania State Medical Society, to confer with Mr. Frank K. Fairchild of the National Selected Morticians Research Corporation, and it is a great pleasure to record here the instant response and hearty and most helpful cooperation of Mr. Fairchild and their consulting chemist, Mr. Jerome Alexander of New York. Several delightful and helpful conferences with these gentlemen have aided us greatly in understanding and appreciating the difficulties of the situation and to make clear to us the very earnest efforts being made by the National Selected Morticians to improve the professional education of morticians and to seek and merit the closest cooperation of the medical profession. We are indebted to Mr. Fairchild and Mr. Alexander for a number of embalming fluids for our studies and especially for the privilege of experimenting with one of their own manufacture to be rendered available for use by undertakers; the proceeds from the sale of which are to be devoted to the promotion of research work on embalming fluids and methods. This fluid contains formaldehyde, glycerin, borax, citrate, sodium chloride, potassium nitrate, benzaldehyde, and alcohol. By a slight modification in their original fluid, consisting of a reduction in the glycerin content and an increase of the alcohol, we have embalmed numerous dogs followed by necropsies at varying intervals and found the fluid, injected in proper amounts according to body weight, capable of preserving the bodies for weeks and even months at room temperature with remarkably slight alterations in the gross and histologic appearances of the internal organs and muscles.

Without detailing the results of our investigations at this time we believe that it is readily and easily possible for the physician to meet all the legitimate objections to the necropsy raised by the mortician either by conducting the necropsy with the technic and courtesies summarized above or by asking the mortician to embalm before the necropsy. If the latter procedure is adopted we are in position to state that *if the undertaker will do his work thoroughly in order to obtain a wide distribution of the embalming fluid throughout the capillary tree, he need not worry at all about preservation* and that for this purpose he may use either his usual embalming fluid diluted one-half the usual strength (with full strength for the head) or one of the newer fluids, like those prepared by Professor Hewson and Mr. Alexander. It is true that the pathologist may encounter the fluid in some of the great vessels and serous cavities but this need not be confusing; in so far as the purposes of the necropsy are concerned its ends may be readily attained and the mortician and friends of the deceased may be assured that the preservation

of the body will not be interfered with, providing the undertaker injects the embalming fluid with a reasonable degree of skill and thoroughness. If the mortician will do his work well and the pathologist conduct the necropsy with reasonable skill and care and be not lacking in sympathy and courtesy toward the mortician, there need not be any excuse for a lack of heartiest cooperation between the medical and undertaking professions, with an increased percentage of necropsies so vitally necessary for the advance of medical science and education.

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DISCUSSION

Dr. Alexander.—There is a movement on foot to make a real profession out of undertaking. I do not need to tell you that the great profession of medicine was once not all that it is today. In the last few decades it has developed most remarkably. Compared with early times, there has been a very remarkable change in funeral customs. In ancient Egypt the embalmers were required by law to report to physicians anything they found out in the course of the embalming process, in order to advance the science of medicine. It is highly important to remember that the care given to the dead has a beneficial effect on the living.

Dr. Kolmer has been put in possession of the facts concerning this movement to make all undertakers cooperate more readily with physicians. I hope that as a newer class of undertakers develops, professional ideals will be more widespread among them, and physicians will find that they will get more useful cooperation from embalmers. I wish to thank Dr. Kolmer for the work he has done in this matter.

Mr. Papello.—The subject has been very well covered by Dr. Kolmer and Mr. Alexander. There is no doubt at all but that the real progressive mortician wishes to cooperate with the physician. There is no reason why any real undertaker should not be glad to cooperate.

Dr. A. S. Giordano.—I wish to congratulate Dr. Kolmer on his paper. When I first came to South Bend I was very much surprised to find the autopsy percentage in hospitals almost zero. Necropsies were opposed by morticians. We got together with the undertakers and the autopsies have increased. I am very much in favor of not having any embalming done before autopsy for very obvious reasons, and, as a matter of fact, embalming can be done more easily and more completely after the autopsy than before. Therefore, to obtain complete cooperation from the morticians, the pathologists must take the trouble to demonstrate this point to his morticians, and then all opposition will cease.

Dr. Margaret A. Miller.—As far as the undertaker is concerned, I should think it would be easier if all of the organs were removed. On the other hand, many times relatives object to having any of them removed. Frequently something is found in sections which might give a solution of the problem if it were possible to reexamine the entire organ, and take sections along the course of the ducts or vessels.

Dr. Eugene A. Case.—If we remove organs when we have been asked not to, we lay ourselves open to the charge of dishonesty. The end sought does not justify the means. The question of the use of embalming fluid is an important one. In an autopsy your findings are based upon the consistency of the organ, its shape, its size, its color, the amount of blood present or the absence of blood, etc., and embalming may so change the appearance of the organs that it will be difficult or almost impossible to form a correct opinion of the pathologic alterations present, this process of embalming destroying much valuable evidence. In certain diseases we may wish to apply special stains to the cells, and as most embalming fluids contain formaldehyde this becomes exceedingly difficult. We are confronted, however, with the condition that whether or not we so desire we must cooperate

with the undertaker; therefore where we find that an autopsy can be secured only by permitting the embalming to be done first, we should permit the embalming.

Dr. T. H. Boughton.—The necessity of not removing organs, together with the obligation of cooperating with the embalmers, led me to develop a very simple method that is applicable in ninety per cent of the cases. With the exception of the brain and the heart, practically any organ can be examined without removing it from the body, simply by lifting it up. It is not necessary to remove any organ or to sever relations with the organ.

Dr. Ralph G. Stillman.—I think it is no more than right that we should pay our respects to the committee of the Pennsylvania State Medical Society for its work with the morticians. When we showed the recommendations made in this report to some of the undertakers and talked it over with them, we found that relations were much improved. So far as the question of autopsy as a whole is concerned it seems to me that it is necessary to work not on the morticians but on the State Legislature. In New York the law has provided that any autopsy shall cease immediately when cause of death is discovered, which taken literally, would terminate many autopsies before they are really completed. It is necessary to remove organs for microscopic examination. It has been impossible to get autopsies where the nearest of kin was at a great distance; the law now provides that the Superintendent of the Hospital may order an autopsy to be performed in the absence of objection on the part of the nearest relative.

Dr. John A. Kolmer (closing).—If the organs are removed the question of their preservation depends a good deal on the period of time intervening between the time of embalming and the time of autopsy. If the autopsy is done immediately it is entirely probable that some of the embalming fluid will escape. The organs are ordinarily returned to the abdominal and thoracic cavities. In regard to the second question, I am sorry that I have no accurate data to draw upon. I am of the opinion that when the necropsy is to be done, it should be done as soon after death as possible, the body in the meantime being kept at a low temperature.

SEDIMENTATION RATE OF RED BLOOD CELLS*

By H. N. Cooper, M.D., Watertown, New York

THE sedimentation rate of the red blood cells is a phenomenon which has attracted but little attention in this country. On the other hand, in reviewing abstracts of foreign journals, especially the German ones, one is impressed with almost weekly references to this test. The startling variations in the rate at which the red blood cells settle down in oxalated blood, when left standing, seems to indicate that definite conclusions could be drawn from this observation, and sufficient work has been done on the test, corroborated by many workers, to place this test in more common usage in all laboratories. Valuable information can be placed at our disposal in many instances.

METHODS

Westergren[1] used a method which has been copied somewhat but which required special syringes and tubes and seemed unnecessarily complicated. Linzenmeier[2] noted the time required for the red cells to settle to definite points. His technic is used in many instances and a recent paper by Baer and Reis[16] of Chicago commends this method. Fahraeus[3] used multiple readings at definite time intervals, which feature I have copied. Zeckwer and Goodell[4] in this country used 8 c.c. blood and 2 c.c. oxalate solution and read at one hour and twenty-four hours. I have modified their technic in two ways: (1) using powdered oxalate, thus avoiding dilution, and (2) multiple readings within the first hour, which seem to have an advantage. Blood is drawn into a bottle into which three drops of 20 per cent potassium oxalate has been allowed to dry. This anticoagulant is sufficient for 15 to 20 c.c. of blood and is used in conjunction, usually, with the taking of a specimen for blood chemistry. The test should be performed as soon as possible, although a delay of a few hours does not materially change the readings. Into a dry 15 c.c. graduated test tube is put 5 c.c. of blood. This is allowed to stand in a rack and readings are taken of the level to which the cells have settled at the following periods: 5, 10, 15, 30, 45, and 60 minutes. The tube is centrifuged at high speed for ten minutes and a reading is taken, which corresponds to the cell volume. This figure is practically identical with that obtained by twenty-four hours' sedimentation. During the latter part of my work, the readings were figured in per cent; thus a cell volume of 2.5 c.c. (of 5 c.c. blood) is 50 per cent. While a confusion of tests is undesirable, I feel that this method is as simple, as free from extraneous influences, and gives as complete a curve as any of the above methods.

*From the Department of Laboratories, House of the Good Samaritan, Watertown, New York.

Read before the American Society of Clinical Pathologists, Philadelphia, Pa., May 22, 1925.

NORMAL STANDARDS

All cases, excepting the four groups below mentioned and those with a red blood cell count below 3,500,000, were used to determine the normal levels. Fifty-eight cases are used in Chart I, showing the range within which these fell. The fastest rate or the lowest line in this group determines the division between the normal and pathologic. The importance of a conservative idea of the normal is obvious. In over one hundred and twenty-five tests every reading below this line was definitely pathologic and could be classed in one of the four groups.

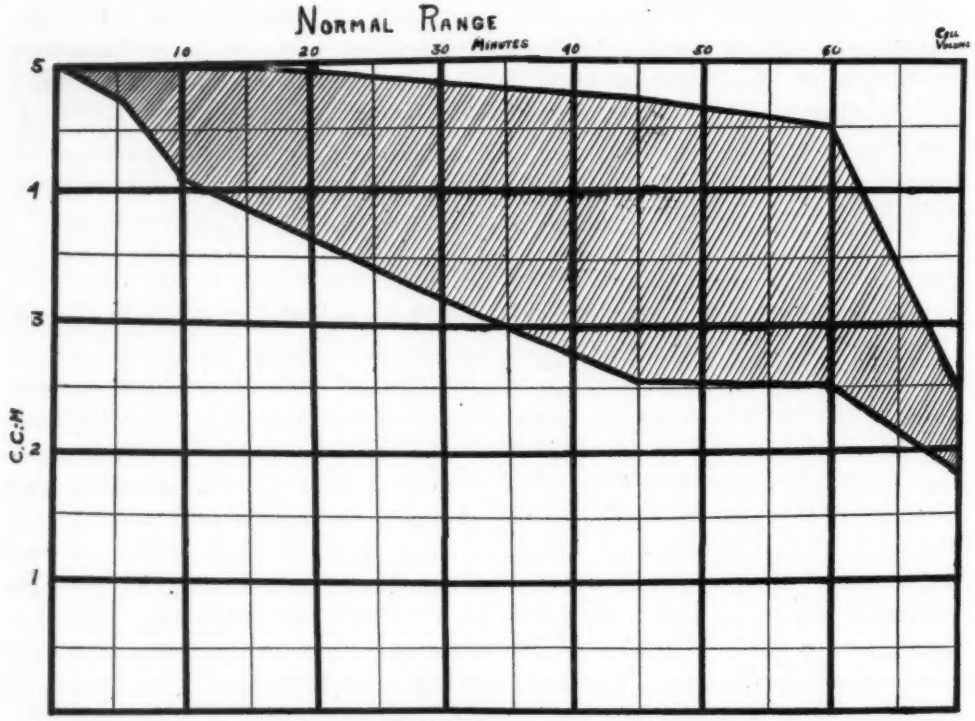

Chart 1

ACUTE INFLAMMATIONS

Chart 2 lists six cases. A definite acceleration is noted in all except line 1, in which case the acute inflammation had subsided, the cause being removed at operation and presumably the rate having returned to normal even before the white blood cell count had subsided. Definite conclusions cannot be drawn from the few cases recorded here, but, in conjunction with other reports, the conclusions are drawn that the line lowers as the leucocytes increase, and a lowering in the early periods corresponds to a high polymorphonuclear count and gives a good prognosis. This rapid fall in the cells in the five and ten minute periods is very striking and, so far in our cases, indicates a favorable reaction. The cell volumes in these cases were nearly normal. Linzenmeier,[2] in reviewing many tests done on gynecologic cases, states that as long as the rate is increased virulent bacteria may be present

in the body, and, hence, he advises delay in operation. He also uses this test to differentiate between acute inflammation of the adnexa and tubal pregnancy, the latter showing no increase. Baer and Reis[16] in a recent and very creditable work, show the value in pelvic pathology of this test, both as a means of differential diagnosis and as a prognostic index.

TUBERCULOSIS

A great amount of work has been done with this test in connection with tuberculosis. Morriss[5] says this test offers a valuable estimation of the degree of activity of this disease. Patients with definite symptoms of activity, fever,

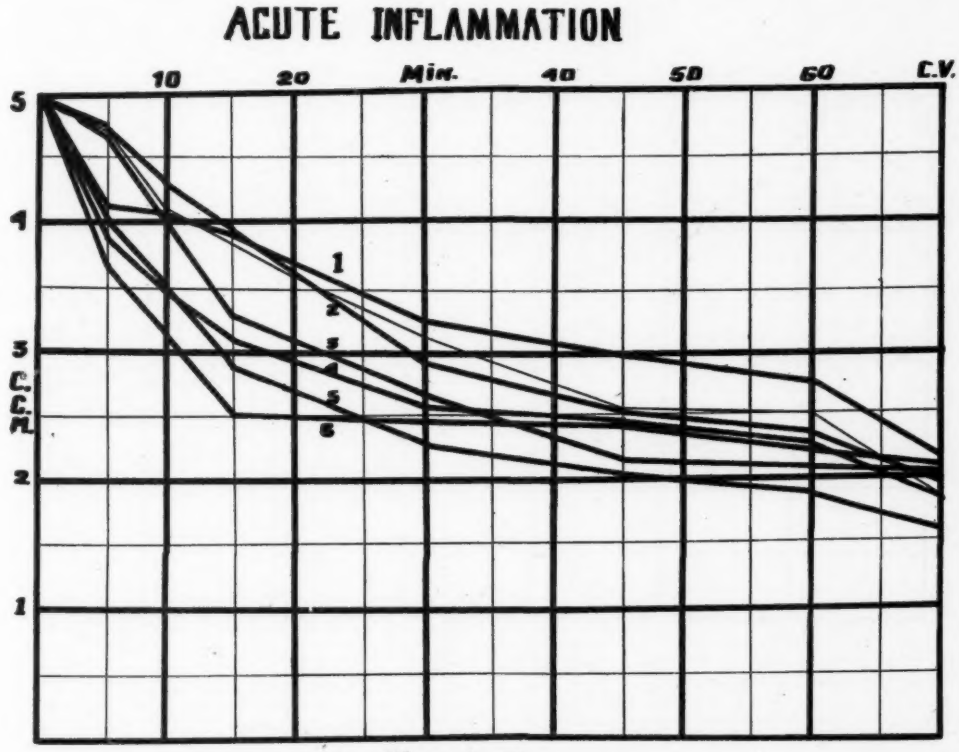

Chart 2

Acute Exudative Inflammations.

No. 1.—Case 61. Acute cholecystitis four days postoperative. W.B.C. 16,000.
No. 2.—Case 36. Erysipelas, first day, blood taken before eruption appeared. W.B.C. 15,000.
No. 3.—Case 43. Abscessed appendix. W.B.C. 19,400.
No. 4.—Case 46. Pelvic abscess.
No. 5.—Case 51. Bilateral pneumonia, empyema, died.
No. 6,—Case 67. Lobar pneumonia, recovered.

and rapid pulse, always give an increased rate. Popper and Kreindler[6] examined two hundred and fifty patients and found that all progressing pulmonary affections show accelerated sedimentation, even with minimal lesions not yet discoverable on clinical and radiographic examinations. Gardère and Lainé[7] made a study which showed a parallelism between a rapid rate and the gravity of the disease. Dreyfus[8] agrees with this and thinks this test is a

better indication of the activity of the process than the temperature cure. Weicksel,[9] however, is cautious in his prognosis except in advanced cases. As shown on Chart 3, minimal cases or those improving show a normal rate, and the worse the involvement the faster the fall in the level of cells. The cell volume bears a constant relation to the patient's general condition. A high value is found in cases progressing favorably. In this our records agree with those of Schlomovitz.[10]

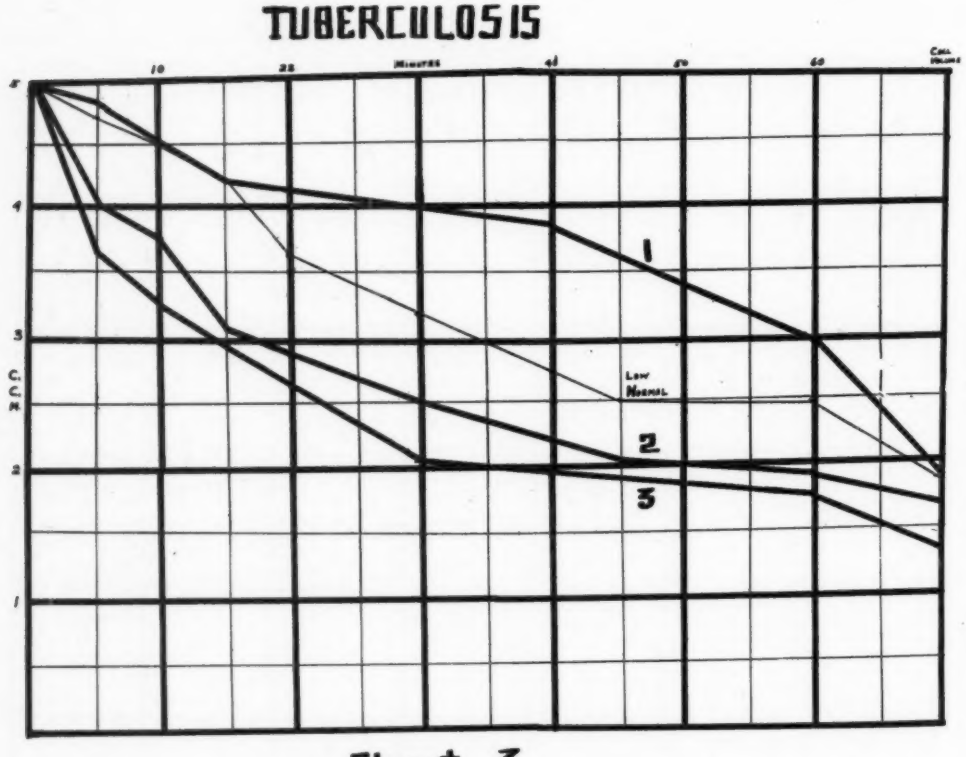

Chart 3

No. 1.—Case 76. Bilateral tuberculous epididymitis two years ago. Osteomyelitis of ribs. Age 83. Condition good.
Case 100. Incipient tuberculosis. Temp. 99.
Case 101. Minimal tuberculosis. County sanitarium.
Case 104. Positive sputum. Gained weight. Improvement.
No. 2.—Case 103. Moderate pulmonary and laryngeal tuberculosis.
Case 102. Moderate pulmonary. Temp. 102.
Case 66. Sputum positive. Temp. 103.
No. 3.—Case 12. Pulmonary tuberculosis, diabetes, died.
Case 98. Advanced sanitarium case.
Case 99. Terminal sanitarium case.

PREGNANCY

Pregnancy shows an increased rate. Pewny[11] shows that during later pregnancy this test will differentiate between that condition and myoma. Zeckwer and Goodell[4] noted that some cases of pregnancy did not have an increased rate. In Chart 4 I have grouped the cases according to the period of gestation and the presence of toxic symptoms. There is a progressive acceleration during pregnancy, and the presence of even slight toxic symptoms, such as morning sickness, increases the rate in that period. One of

the fastest rates in this series of cases is shown in line five; it is a combination of pregnancy, toxemia, and acute inflammation. Briefly, the patient presented on admission the picture of eclampsia, acute nephritis with complete suppression of urine, delivered of a dead fetus, high blood pressure and a rapidly increasing nitrogen retention of the blood. A decapsulation of one kidney was done and following this, urine began to be excreted in increasing amounts. The sedimentation test showed a marked acceleration on the day of her operation. The fastest rate was on the day she started excreting urine.

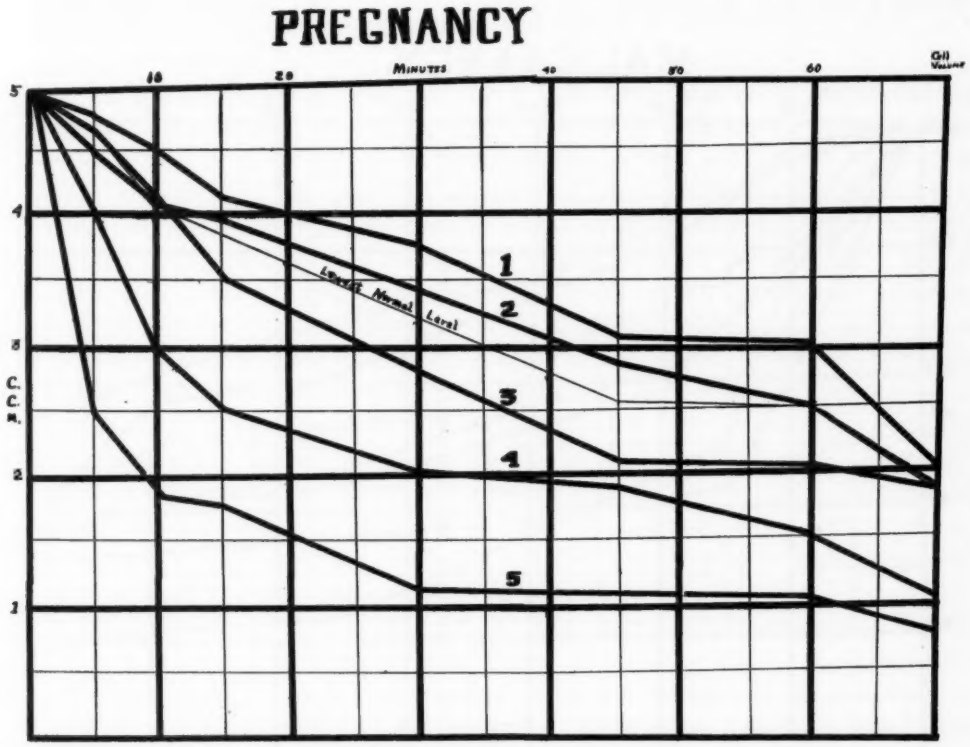

Chart 4

No. 1.—Average of seven cases. Normal pregnancy, three to seven months.
Case 29. 3 months pregnant, normal.
Case 41. 6 " " "
Case 84. 4 " " "
Case 87. 4 " " "
Case 78. 7 " " "
Case 91. 6 " " "
Case 92. 3 " " "
No. 2.—Average of three cases, showing early toxemia.
Case 79. Nausea persisting for six months.
Case 85. Nausea, four months pregnant.
Case 86. Five months, nausea and slight edema.
No. 3.—Normal pregnancy over seven months.
Case 33. Normal pregnancy at term.
Case 49. Normal at eight months.
Case 60. Nine months, normal except for four-plus Wassermann.
Case 89. Normal eight months pregnancy.
Case 90. Normal seven months pregnancy.
No. 4.—Late pregnancy, moderate toxemia.
Case 42. Nausea persisting, edema, urine normal.
Case 93. Urine shows trace of albumin, blood pressure 160/85.
Case 97. Albumin in urine, blood pressure 145/90, labor induced.
No. 5.—Eclampsia, acute suppression of urine, N.P.N. 170 mg., creatinine 6.6, cholesterol 532. This line is the lowest line (fastest rate) in the series and was taken at the highest point of the disease. Three other readings showed a slower rate when the disease was less severe. Patient recovered.

Her nonprotein nitrogen rose for a week after this, reaching 170 mg. per 100 c.c. blood, but at this time her sedimentation rate had started to improve. In four weeks both her rate and her chemistry were normal. From a prognostic standpoint, her sedimentation rate was the earliest laboratory procedure to demonstrate improvement. Improvement in blood chemistry is about a week behind clinical symptoms.

MALIGNANCY

The change of stability of the red cells in the presence of malignant growths has been universally recognized. Roffo[12] and Zeckwer[4] both recog-

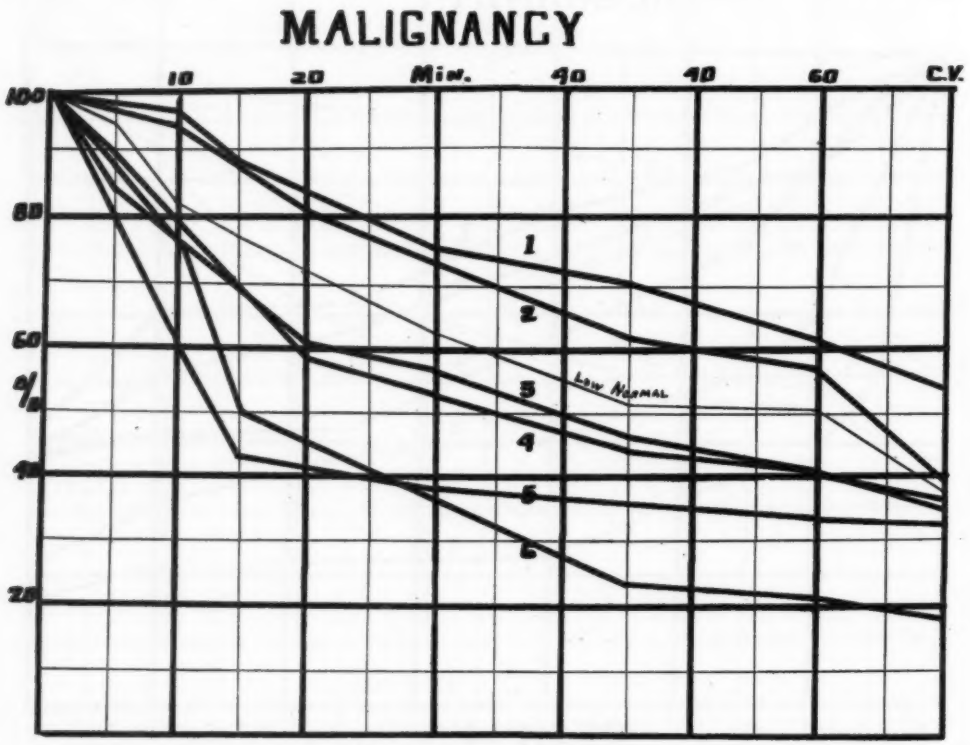

Chart 5

No. 1.—Case 15. Intraligamentous uterine fibroids.
No. 2.—Case 96. Early superficial carcinoma of tongue.
No. 3.—Case 63. Adenocarcinoma of breast, 6 cm. dia. No metastases.
No. 4.—Case 19. Carcinoma of stomach, "Linitis Plastica."
No. 5.—Case 59. Chorioepithelioma, early, general condition good.
Case 32. Papillary carcinoma of ovaries, with multiple peritoneal metastases. Sedimentation rate practically identical with Case 59.
No. 6.—Case 65. Lymphosarcoma involving cervical, axillary, inguinal and mesenteric (primary) nodes. Aged 63. Diabetes for two years. R.B.C. 1,760,000, Hgb. 45 per cent.

nized the great increase in rate in rapidly progressing tumors and the slight increase, or no increase, in small, localized ones. Chart 5 records the rate in seven cases. A benign myoma showed no increase. An early carcinoma of the tongue was within normal limits. Increasing rates were found, lines 5 and 6 showing the rates on highly malignant and rapidly fatal types. In this group also the cell volume shows the general condition of the patient. Thus the degree of malignancy can be measured.

GENERAL CONSIDERATIONS

The causation of this phenomenon has been attributed to two factors: (1) Increased fibrinogen content of the blood. As evidence in support of this theory blood which shows a rapid rate when drawn, shows a slow normal rate when defibrinated. This is contrary to the findings of some observers, but it was definitely our experience. (2) Changes in the albumin and globulin ratio of the plasma. Hober and Mond[13] confirm Fahraeus' theory, which has to do with negative electric charges being great in albumin solutions, less in globulin and least in fibrinogen, the red cells carrying the charge, in this regard. Hence, when the charge is least, the cells repel each other less, and settle down more rapidly.

An additional factor that may enter into this causation is the cholesterol content of the blood. The hypercholesterolemia is well known in pregnancy and cancer. In this series cholesterol determinations were done on the majority of cases. In all cases of accelerated rates there was an increase in cholesterol above the normal. The importance of this determination is being recognized now, especially in degenerative kidney lesions or nephrosis. All observers agree that the sedimentation rate is increased in any disease attended by tissue degeneration and destruction. Experiments were attempted to prove this theory, but, because of the insolubility of cholesterol in watery solutions, no results have been obtained up to the present. MacAdam[14] concludes in his genitourinary work that a low cholesterol signifies low capacity for antibody formation, gives a bad prognosis, and is a bad surgical risk. Leupold[15] analyzed twenty cases of infectious diseases and showed a rise in cholesterol during convalescence. Presumably these cases would have shown an increased sedimentation time. Thus the hypercholesterolemia is well recognized and is at least a concomitant feature in these cases.

The cell volume or the hematocrit test furnishes interesting data. If read in per cent, and using the first figure as in the millions, it corresponds to the red blood count. Thus normally the cell volume is 50 per cent or 5,000,000 red blood cells. In all our cases, the greatest variation found was a difference of 600,000. The cell volume is a better indication of the condition of the corpuscles than a count. For example, a count of 4,000,000 red cells of the microcyte type will give less than 40 per cent volume. Hence it gives a true index of the total volume of red cells or the total hemoglobin capacity of the cells. It corresponds in a way to the color index. A marked secondary anemia showing a low cell volume would, of course, indicate the poor physical condition of the patient. Thus the cell volume shows the general physical condition and the real systemic extent of the disease.

CONCLUSIONS

1. Increased rate in sedimentation of the red cells occurs in diseases attended by tissue degeneration and destruction. Principally this included: (1) acute inflammations, (2) pregnancy, (3) tuberculosis and (4) malignancy.

2. The curve during the first hour, and the final cell volume, offer prognostic and diagnostic value.

3. The cause of the increased rate is associated with a hypercholesterolemia as well as an increase in globulin and fibrinogen in the blood.

I wish to acknowledge my thanks to Dr. Page Thornhill, Chief of the Pre-Natal Clinic and to Dr. H. L. Smith, Director of the County Tuberculous Sanitarium, for their aid in securing material for this work.

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DISCUSSION

Dr. Ward Burdick.—I know nothing about it, but I have noticed this phenomenon several times while doing fragility tests, not knowing how to interpret it. I am very glad to have heard this paper. It apparently opens up a field full of promise.

Dr. S. L. Leiboff.—We have obtained similar results to those of Dr. Cooper and in our technic we simply use one c.c. of blood instead of ten. We found that by using 1 c.c. of blood we get just as good results. We read the tubes at five minutes, ten minutes, fifteen minutes, half an hour, and two hours.

Dr. Robert A. Kilduffe.—I think this is an extremely interesting paper and one which I had hoped to see more freely discussed.

This method of investigation is applicable to and of interest in a great variety of conditions, but, in order to have conclusions of value drawn, some idea of the normal variations must be had.

How is this to be obtained, however, if everyone using the test employs a different method? The method described has the advantage that the specimen may also be used for blood chemistry.

I think it would be very important and of value if the members of this Society would investigate different methods and the use of different amounts of blood so that we could determine, first of all what was a satisfactory amount of blood, and then, perhaps, using one method, we could get figures which would be comparable and of definite value.

Dr. George D. Fussell.—I would like to ask if an anemia does not alter the reading a great deal, regardless of the cause of the anemia.

Dr. H. N. Cooper (closing).—As I mentioned, everyone is using a different method, each seeming to favor his own modification. In regard to the reasons why I did not use Westergren technic: first, the syringes, etc. are hard to obtain and, second, I feel inclined

not to favor small tubes, anything less than 5 mm. in diameter, as capillary attraction and other elements enter into the reaction in such cases. A large tube, it seems to me, is more desirable. The reading, as indicated in the early part of the first hour, has a great deal of significance, and, while other observers have used frequent readings, the majority have read at hourly periods. Diluting the blood with oxalate solution may have something to do with the sedimentation rate. This test obviates that factor, and, further, the blood can be used later for blood chemistry determinations. Anemia does not increase the rate. There is no doubt that a standard test would be desirable, and I think it would be a splendid thing for this Society to establish a standard for this test.

SUGGESTED METHOD TO BE FOLLOWED IN DEVELOPING A STANDARD COURSE FOR MEDICAL TECHNICIANS*

By Walter E. King, M.A., M.D., Detroit, Michigan

THE clinical laboratory is more than a workshop involving mere matters of routine. It represents a clearing house, to which is referred clinical materials of widely diversified nature. From the studies of the materials subjected to examination, data are obtained which are utilized by the physician. As Woolley has stated, "By means of such data the physician is more easily able (or more quickly able) to arrive at a diagnosis, to estimate accurately a prognosis, and to institute confidently logical therapeusis."

In efficiently conducting the various analyses literature must be consulted, judgment must be exercised, experience and training must be called into action. In the small clinical laboratory, under the supervision of a visiting pathologist, as well as in the larger, well-organized laboratory directed by a resident clinical pathologist, one or more individuals are called upon to do this work. These individuals are regarded as medical technicians. A medical technician is one who is able to conduct clinical laboratory tests, examine clinical materials, and assist the pathologist and the physician in carrying out various diagnostic procedures.

Heretofore, relatively scant attention has been given to the part which the medical technician takes in the diagnosis of diseases. The technician is, of course, required only to report various findings to the pathologist; such work, however, carries with it a great responsibility. Unless the technician's work is carried out intelligently and carefully the clinician's diagnosis is in danger of being inaccurate. Just as the physician's clinical diagnosis should be based upon thorough scientific observation, resting upon a background of honesty of character and knowledge of medicine, so must the laboratory reports be based on careful and intelligent work where reliability is vouched for by a trained technician.

The purpose of the laboratory is to discover facts which will help the physician and benefit the patient. No degree of skill, knowledge, experience, or brilliancy of any laboratory director, or clinical pathologist can entirely

*Read before the Fourth Annual Meeting of the American Society of Clinical Pathologists, Philadelphia, May 20 to 23, 1925.

From the Medical Research and Biological Laboratories, Parke, Davis and Company, Detroit, Michigan.

compensate for lack of skill, scanty knowledge, inexperience, or stupidity on the part of the technician.

At the present time, the maintenance of laboratory facilities or access to such has become a necessity to every community. Hospitals, clinics, physicians, individually and in groups, sanatoriums, asylums, private and industrial laboratories, and state and municipal boards of health laboratories must be supplied with more and better trained technicians. The work which these individuals perform is as exacting and as highly professional in nature as that of the registered pharmacist or the registered nurse.

Fifty years ago the clinical laboratory, as an important diagnostic aid, did not exist. Today we are confronted with the problem of the training of individuals for this comparatively new profession. We must, therefore, keep in mind, first, the rapid development of medical technology and, second, the rapidly growing demand for more and better trained men and women in this vocation.

A variety of methods has been followed in attempting to train laboratory technicians. The most common procedure has been to train the worker individually. One frequently hears a laboratory director state that he prefers to train his own technicians. Such training must necessarily be relatively narrow and result in too great a degree of imitation. Unfortunately some technicians who might otherwise have been good students are spoiled by this method and become inefficient, automatons rather than broadly trained, helpful assistants.

Too many of our prominent clinical pathologists and physicians have voiced the statement that any experienced laboratory man can train technicians to do the routine work. The director of a busy laboratory has little available time to devote to the development of his assistants. Even in the presence of the leaders among the American clinical pathologists, one may boldly make the statement that not all clinical pathologists are teachers. Perhaps at a somewhat safer distance one might venture the remark that few clinical pathologists possess the patience, the love of imparting knowledge, and the passion for stimulating development that are required of the successful teacher.

There are many who believe that the college or university course offers all that is necessary for the student who desires medical technology as a vocation. This method of training has been tried for many years. Regardless of the courses which are elected, the student finds himself at the end of the college or university career almost totally lacking in practical knowledge and laboratory experience in the most commonly required diagnostic procedures. This criticism applies, to a very considerable extent, even to those universities which offer certain courses recommended as leading to service as clinical laboratory technicians. Making proper allowance for the broadening influence and the added advantage of college education for any line of work, however, one may observe almost as many failures among technicians who are college graduates as among those who have attempted to pick up training. The reason for this lies in the fact that the successful medical technician needs not only an intimate working knowledge of the various subjects involved, but also requires *a correct viewpoint of medical technology as a pro-*

fession. The student should secure a broad foundation, learn well the many different methods of technic, become proficient in scrutinizing newer developments, understand the interpretations of laboratory findings, and learn to appreciate the doctor's problems. In other words, *adequate preparation for this work involves professional training.*

There has rapidly developed a necessity for a prescribed course of study consisting of didactic teaching, laboratory exercises, and practical training, all carried out according to strict pedagogical methods. The ideal training for medical technicians should consist of a four year course of study leading to the degree of B.S. in medical technology. As has been stated, such a course cannot be secured advantageously by selecting subjects here and there from different departments of the university or medical school. It is essential that such a course should be strictly technical or professional in which a large proportion of the work is that specifically involved in medical technology.

To develop the proper course of training for medical technicians the work must necessarily pass through transitional stages. The extent of demand for technicians, the perfection of new methods of laboratory diagnosis, as well as other factors, would materially influence the trend of such development and the period of time necessary to reach the point when a standardized course of training might be realized.

In the plan which is outlined, it is assumed that this project would be undertaken under the direction of a medical school, preferably one which is a part of a large university. The institution should be closely affiliated with hospital facilities and, if possible, with the local state Board of Health laboratory.

Such would be the ideal arrangement. Any educational institution of good standing, however, could put into practice the plan which is submitted and thereby fulfill a distinct service to the medical profession and to humanity.

The courses which are outlined would be subject to considerable change, as the aim should be to give proper balance, emphasizing those subjects most demanded of the laboratory technician. No diagnostic procedures should be omitted. Those who are interested in teaching these students find it necessary to make frequent and sometimes radical changes in the texts of laboratory directions in order that proper instruction may be maintained. The student should be impressed with the rapidly changing methods of the laboratory field and should possess a training broad enough to recognize and adopt newer methods of procedure which are of value. This constitutes one of the most important factors in the successful training of the student technician and establishes in a definite manner the need for a well balanced, pedagogically conducted, technical course of study for clinical laboratory workers.

Conservative judgment would indicate that a school for student medical technicians, which would fit those capable of doing accurate work and of growing in the field of endeavor, could be well developed within a period of from three to five years.

It would seem best in the beginning to offer only a vocational course. This could be extended as rapidly as conditions would justify, and within a few years the institution, which might undertake this project, should offer a four year course carrying with it a Bachelor's degree for those who might

desire the full college course, and a two year course, vocational and technical in character, not leading to a degree, for those wishing to qualify as certified routine workers only.

Courses of study should be arranged under three divisions, the three divisions representing stages in the development of a standardized course of study for medical technicians. Stage one, which might be undertaken in the beginning and which would consist of a full one year course, should occupy for its development a period of only one year, certainly not more than two years. Stage two, consisting of a full two year course, should be well begun during the third year after the beginning of the project. Stage three, representing a four year course, leading to the Bachelor's degree, could be instituted at any time that conditions might justify.

In the following suggested plan, it is assumed that all routine laboratory diagnostic procedures are included under the various courses listed. For instance, the routine examination of spinal fluid is included under "clinical analytical methods," "serology," and "hematology."

The various courses submitted are suggested as a basis upon which to build. As the work progresses, some additions and considerable rearrangement probably would be found necessary or desirable.

The outline here submitted is the result of three years of intensive study and practical application in the training of approximately one hundred and fifty student technicians.

STAGE I

Prerequisite: High school course, from schools accredited by the Association of American Colleges.

One Year Course, covering a period of nine months (36 weeks or 1080 college hours) of didactic training, followed by four months (16 weeks, 8 hours per day, approximately 700 working hours) of apprenticeship or practical training in an accredited hospital or public health laboratory.

The one year course may be arranged as follows, and cannot include instruction in roentgen ray or basal metabolism except through a few "special lectures."

Arrangement of Courses:

SUBJECT	LECTURE HOURS	LAB. HOURS	LAB. PERIODS (4 HR.)
Clinical Bacteriology	48	136	34
Clinical Analytical Methods	48	120	30
Hematology	30	120	30
Serology and Immunology	34	148	37
Blood Chemistry	20	40	10
Urinalysis	30	80	20
Parasitology	15	8	2
Tissue Technic	5	40	10
Functional and Sensitization tests	3	28	7
Anatomy and Physiology	40		
Medical Terminology	40		
Interpretation of Findings	10		
Autopsy Demonstrations	10		
Special Lectures	27		
Total	360	720	180
Apprenticeship (16 weeks, 8 hours per day)		704	
Total laboratory		1424	

STAGE II

Prerequisite: High school course, from schools accredited by the Association of American Colleges.

Two year course, covering two nine month (36 weeks or 1080 college hours) periods of didactic training, with two four month (16 weeks, or approximately 700 working hours) periods of apprenticeship or practical training in an accredited hospital or public health laboratory.

SUBJECT	LECTURE HOURS	LAB. HOURS	LAB. PERIODS
Clinical Bacteriology	72	200	50
Clinical Analytical Methods	72	200	50
Hematology	48	180	45
Serology and Immunology	60	220	55
Blood Chemistry	32	80	20
Urinalysis	32	88	22
Parasitology	20	20	5
Tissue Technic	18	80	20
Functional Tests	10	20	5
Anatomy and Physiology	72	8	2
Sensitization Tests	6	4	1
Roentgen Ray	120	240	60
Basal Metabolism	26	100	25
Medical Terminology	72		
Interpretation of Findings	10		
Autopsy Demonstrations	10		
Special Lectures	40		
Total	720	1440	360
Apprenticeship (32 weeks, 8 hours per day)		1408	
Total laboratory		2848	

It is suggested that this course be divided as follows:

First Year:

SUBJECT	LECTURE HOURS	LAB. HOURS	LAB. PERIODS
Clinical Bacteriology	72	220	55
Clinical Analytical Methods	72	220	55
Hematology	48	180	45
Urinalysis	32	88	22
Anatomy and Physiology	72	8	2
Sensitization Tests	6	4	1
Medical Terminology	50		
Special Lectures	8		
Total	360	720	180

Apprenticeship (16 weeks in urinalysis, blood, and general routine work including clinical bacteriology).

Second Year:

Remainder of subjects and completion of apprenticeship (16 weeks) emphasizing Roentgen ray, blood chemistry, serology, and basal metabolism.

STAGE III

Prerequisite: Same as above.

Four year course, leading to Bachelor of Science degree in Medical Technology. This course should consist of the two year technical course presented above, preceded by two years of college work. The college work should consist of the accepted premedical course of sixty semester credits of college work, including English, six credits; Chemistry, thirteen credits; Physics, eight credits; Biology, six credits; and French or German, six credits. Subjects suggested or advised are Latin, English, Mathematics, Psychology, Sociology, Physiology, Comparative Anatomy and Drawing.

It can be seen that such a scheme, with such modifications as conditions required, could be undertaken without difficulty at any well organized educational institution. Insurance against failure would be afforded by a natural evolution of the training from a one year vocational course to a four year college course. Students successfully completing such courses, trained in an academic atmosphere, inspired with true scientific spirit should promptly find a large measure of usefulness.

Such a project, successfully carried out, would prove of inestimable assistance in the standardization of clinical laboratory technic and personnel. As soon as one educational institution should have demonstrated the value of such an undertaking, others would surely follow. The results of such work would, in a very few years, definitely improve the professional character of the medical technician and materially assist in raising the standard of the clinical laboratory.

DISCUSSION

Dr. Robert A. Kilduffe.—I think we all realize that this is a very important subject and a very important paper. It is impossible for any one to run a laboratory of any size without technicians and a good technician is a pearl beyond price. In order that the supply shall meet the demand there must be some source from which the supply shall come and this source Dr. King has endeavored to furnish. I think that Dr. King has outlined a very excellent and thorough course and I am in entire accord with him except for one thing: I do not think it either necessary nor advisable that technicians shall be instructed in the interpretation of the examinations they may make or help to make.

The word technician implies that their training shall be mainly in things technical. I demand of a technician, first of all, that there shall be no stalling. If he does not know how to do what I tell him to do, I do not want him to try and work it up out of a book. I like a technician to know why he does what he does, because I believe that this information leads to better and more careful work.

I want him to know that there is a definite reason behind the making of a blood count, but I do not think it necessary that he shall be taught the elements of blood count interpretation. This, it appears to me, is impossible, because for the intelligent interpretation of laboratory results much more is required than a smattering of information. I think that if technicians are led to believe that these interpretations are largely rule of thumb matters, which, of course, is far from the truth, the next step is the self-graduation of that technician into what he thinks is a clinical pathologist.

I think technicians should be trained to observe. I believe that a properly written report of a sputum examination, for example, should include some statement as to the gross characteristics and appearance of the specimen. I believe such observations properly constitute a part of the examination of the specimen.

I am opposed, however, to endeavoring to explain to technicians the interpretation of laboratory findings. I do not believe that it can be done in a short time, without a thorough preliminary training in a variety of subjects. I think that the main difference between a technician and a pathologist is the ability to interpret in terms of the patient and his condition the laboratory findings, and for this much more than mere technical training is required.

Dr. A. H. Sanford.—I believe that Doctor King's paper is worthy of further consideration and attention by this Society, perhaps next year. It is in many ways an important and as vital a subject as the standardization of laboratories. Technicians are part of the working material, part of the equipment. The University of Minnesota offers a course in medical technology leading to a B.S. degree. I have had girls who have had this course, and they were very efficient. Some others may not be so good. Some of the girls may get interested in medicine and later, as physicians, become clinical pathologists. Many of the young women applying to me for positions are school teachers, and they

often make good technicians. Another group is made up of registered nurses; they have to start back at the bottom, and occasionally they are willing to make the financial sacrifice. Still another group includes the bright high school girls, dexterous and willing to work. They are limited on account of lack of education, but they constitute a valuable and necessary part of a laboratory staff.

The "experienced technician" presents another story; she keeps flitting from tree to tree, with an increase in salary for every flit. The really good technician is a "pearl without price," and is often hard to keep. Medical technology as a profession offers a real field of endeavor for the educated woman. If she loves the work she will get a remuneration that is fair, and as a life work she will usually like it better than teaching.

Dr. A. S. Brumbaugh.—Doubtless you are all familiar with the psychologic tests for mental efficiency now commonly used by educators, and those who undertake to teach technicians should examine their mental caliber and perhaps give some test of that character. Those who do not measure up fairly well should be dissuaded from taking up this line of work.

Dr. Robert A. Keilty.—If the girls spend five or six years at college they are not going to do the ordinary routine at a low salary. I prefer to train them myself in my own way.

Dr. Walter E. King (closing).—1. Concerning the question as to whether the course which has been suggested is unnecessarily long or represents training which the average technician does not need, it seems to me there can be no argument. I cannot conceive of the idea of withholding knowledge from any student in any line of endeavor. The worthwhile student will seek the light and obtain knowledge regardless of obstacles. The technician's work involves such a diversity of knowledge and such a variety of manipulations and at the same time is so frequently being augmented by new methods that the student can scarcely reach the end.

2. The question as to the salaries of technicians can be answered without difficulty. The salary which the average well trained technician can command justifies a course of college training such as has been suggested. The salary of the technician compares favorably with the salary which is paid the average teacher.

3. It is recognized that there is chance for argument relative to the wisdom of teaching the interpretation of clinical laboratory findings to the student technician. It must be admitted that there is, at present, a tendency on the part of the hospitals to carry on the work of the clinical laboratory without the services of a medical director of the laboratory, depending entirely upon the findings and interpretation of findings by the lay technician. This imposes a responsibiity upon the lay technician for which he is totally untrained as he possesses no knowledge of clinical conditions. This condition of affairs is unjust to the patient and to the medical profession. I hope that I may be understood in attempting to express my opinion regarding the teaching of the interpretation of findings. I do not think that the lay technician should interpret findings and sign reports. The point which I do make, however, and which is clear to me is that for the successful teaching of the technician some stress must be laid upon the interpretation of findings and the application of various findings to clinical conditions. Such a course enables the instructor to fascinate the student in the entire work, to secure the undivided interest of the student and to turn out a much better finished product. Exhaustive study of laboratory interpretations is not necessary. It should be carried only as far as is necessary to hold the interest of the student and to satisfy him. Moreover, this procedure enables the student to appreciate the importance of his work and impresses him with the necessity of the most careful and painstaking effort. It seems to me that this idea of keeping the technician in the dark as to the significance of his findings accounts for the fact that many of these workers remain nothing more than routine drudges upon whose work no dependence can be placed. The lay technician should not be given the responsibility of reporting results and interpreting these results to the clinician. On the other hand, for teaching purposes and in order to develop the student into a highly trained technical assistant, such information should be freely given.

In conclusion, please allow me to state that in presenting these suggestions relative to the problem of supplying the proper course of instruction for laboratory technicians. my ideas have been the result of earnest consideration and intense interest.

FIELD OBSERVATIONS IN SCARLET FEVER*

By F. M. Huntoon, M.D., John A. Murphy, M.D., and S. H. Craig, M.D.
Glenolden, Pa.

THE work reported here was undertaken with the plan of testing different scarlet fever streptococcus toxins on the same persons, of studying its keeping quantities, and of checking the results of immunizing treatment as regards the elimination of the skin test.

The accompanying charts show the results obtained. In reading the results of Dick tests and retests, all reactions were measured in centimeters, and an area of redness of one centimeter was adopted as the minimum positive reaction. Intensity of redness was not included in the standard set for primary tests, but was noted in retests.

Heated toxin controls were used in the early part of the work. They were discontinued for the reason that only a little over one per cent of pseudo-reactions occurred.

Table I is a composite chart confirmative of similar work done by other observers. Its most interesting point is that practically ten per cent of those giving a history of previous scarlet fever had positive reactions to the scarlet fever streptococcus toxin.

Table I

Dick Test Results

Ages	No. Tested	% Positive
All Ages	2796	28.7
Age 1-10	474	47.4
Age 11-20	1717	26.0
Age 21-30	436	25.4
Age 31-40	151	8.6
Age 41-50	18	33.3
History of Scarlet Fever in Above Group		
All Ages	139	9.3

* Two toxins from different sources were used, and some cross reactions were noted, in that a patient would be positive to one toxin and negative to the other. These cross reactions were recorded against both toxins, there being no constancy observed for any one toxin.

Table II shows a comparison between two toxins similarly prepared and is a composite chart.

While there is a difference in favor of toxin *A*, it must not be accepted that toxin *A* will consistently outpoint toxin *B*. The Drs. Dick[1] have recently pointed out the variation in the toxin production, between strains of scarlet

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From the Mulford Biological Laboratories.

fever streptococcus, stating the toxin production of any one strain is fairly constant. Our experience has been that two toxins, similarly prepared, will show some difference in percentage results, in favor of either toxin, in different groups tested.

TABLE II

DICK TEST

COMPARISON OF TWO TOXINS ON SAME INDIVIDUALS

(Both toxins freshly diluted unpreserved)

TOXINS	NO. TESTED, AGES 1-20	% POSITIVE
A. Hyg. Lab. Control Toxin	685	36.3
B. 1050 Toxin		31.2

(St. V.—G.M.—Mor.)

Tables III-*A* and III-*B* are concerned with the keeping qualities of scarlet fever streptococcus toxin diluted for the Dick test. The effect of preservative, temperature, age and method of preparation were studied. The groups tested are too small to aid in a definite conclusion and only serve as indicators.

TABLE III-*A*

KEEPING QUALITIES OF SCARLET FEVER STREPTOCOCCUS TOXIN DILUTED FOR THE DICK TEST

TESTS ON SAME INDIVIDUALS

No. Tested	Freshly diluted unpreserved Hyg. Lab. Control Toxin	73708 Toxin Unpreserved 14 days at 37° C.
20	Positive 15%	Positive 15%
19	Same as above Positive 21%	Same—Preserved 0.5% Phenol 14 days at 37° C. Positive 10%
21	Same as above Positive 19%	Same—Preserved 0.5% Phenol Ice Box 14 days Positive 23%

(G.M.)

TABLE III-*B*

KEEPING QUALITIES OF SCARLET FEVER STREPTOCOCCUS TOXIN DILUTED FOR THE DICK TEST

No. Tested		
61	73708 Toxin Unpreserved Freshly Diluted % Positive 29	73708 Toxin Unpreserved Aged 30 Days % Positive 18 (St.V.)
46	73708 Toxin 0.5 Phenol Aged 30 Days % Positive 15	Same as above % Positive 50 (St.V.)
20	63 Toxin Purified Unpreserved Aged 50 Days % Positive 50	Hyg. Lab. Toxin Unpreserved Freshly Diluted % Positive 50 (U.P.)
157	63 Toxin Purified Unpreserved Aged 56 Days % Positive 19	Same as above % Positive 18 (T.)

Based on the results obtained, the indications are that:

1. A freshly diluted unpreserved toxin and an unpreserved toxin, kept at 37° C. for fourteen days are comparable.

2. The addition of preservative, under the same condition, is harmful to the toxin.

3. The addition of preservative, under cold storage conditions, is not harmful.

4. The same toxin unpreserved, freshly diluted and aged thirty days, shows an advantage for the freshly diluted toxin.

5. The same toxin aged 30 days, preserved against unpreserved, shows an advantage for the unpreserved toxin.

6, 7. A purified, unpreserved practically two-month-old toxin is comparable to an unpreserved freshly diluted toxin. This indication is supported by Table IV-*A*.

All our work in immunization was done by employing a series of three doses, 125, 250 and 500 skin test dose as originally recommended by Zingher.[2]

We have never had with this dosage a severe, general or local reaction.

Other workers, it is true, are employing much larger dosage, with an increase in the number and intensity of reactions produced.

It is questionable whether the use of the increased dosage is advisable, on account of the psychologic effect produced by severe reactions. This method, to be useful, must be applied as a public health measure, and in most communities, such severe reactions will block any general use of the method.

TABLE IV-*A*

IMMUNIZATION RESULTS AT VARYING PERIODS

125-250-500 SKIN TEST DOSES			
Period	No. Tested	63 Toxin Unpreserved Aged 56 Days % Negative	Hyg. Lab. Toxin Unpreserved Freshly Diluted % Negative
20 Wks.	13	69	61.5 (T.)
16 Wks.	59	*Same as above % Negative 90	Same as above % Negative 90 (Mor.)
8 Wks.	20	Same as above % Negative 80	Same as above % Negative 80 (Mor.)
9 Wks.	45	73708 Toxin, Purified, Unpreserved Freshly Diluted % Negative 97.7	Same as above % Negative 97.7 (S.)

*250-500-1000 Skin Test Dose.

In this table a purified, unpreserved aged toxin and a purified unpreserved freshly diluted toxin gave consistent results against the control toxin.

With the doses recorded here, there is no outstanding difference between them.

The immunization results shown compare favorably with other workers. Nesbit[3] in a series of 328 retests reported 65 per cent negative. In a personal

communication, Dr. Nesbit stated he would report later on two more series, using 2000 and 4000 skin test doses as the final dose.

The table indicated a higher percentage of negatives in the early weeks following immunization.

Reports from the Pennsylvania Soldier's Orphans School, Scotland, Pa., and the Pennsylvania Training School at Morganza, Pa., to the effect that three epidemics of scarlet fever had abruptly ended following the first immunizing dose given to the positive contacts, led us to check the time of disappearance of the positive reaction after immunization.

Tables IV-*B* and IV-*C* show the results.

TABLE IV-*B*

IMMUNIZATION RESULTS AT VARYING PERIODS

Retests with Two Toxins

	3 doses completed		2 doses completed	1 dose completed
	4 Wks.	12 days	5 days	4 days
No. Tested	72	37	8	4
% Negative	59	81	38	50

(Mor.)

TABLE IV-*C*

IMMUNIZATION RESULTS AT VARYING PERIODS

(125-250-500 Skin Test Doses)

Retests with Two Toxins

Interval after final dose	1 Wk.	2 Wks.	3 Wks.	4 Wks.	5 Wks.
No. Tested	6	24	36	32	41
% Negative	50	58.3	52.7	43.7	43.9

(U. P.)

The above results indicate that following the immunization a fairly large percentage show an early disappearance of the positive reaction.

In general, those still showing reaction show a diminution in intensity. There are some exceptions to this rule.

Furthermore, some individuals showing an early disappearance of the reaction again become positive within a few weeks.

The fact that in three instances in institutions epidemics have stopped short after the first immunizing injection (and this is supported by personal communications of instances in private practice) taken in conjunction with the early disappearance of the skin reaction in many individuals, indicates that the use of the toxin injections as a valuable emergency measure, regardless of the ultimate result in producing a permanent immunity.

Time only can decide the question of the permanent immunity produced.

Table V shows that a toxin prepared with and without preservative and aged eleven weeks has practically the same immunizing value.

TABLE V

IMMUNIZATION RESULTS WITH A PRESERVED AND UNPRESERVED TOXIN AFTER 11 WEEKS

	RETESTS WITH TWO TOXINS	
	73708 Preserved	73708 Unpreserved
No. Retested	19	18
% Negative	48	45

(C.B.)

TABLE VI

RETESTS ON A PREVIOUSLY NEGATIVE GROUP

	73708 Toxin Unpreserved, Freshly Diluted	Hyg. Lab. Toxin Unpreserved, Freshly Diluted
No. Retested	127	127
No. Negative	122	122
% Changed	4% Positive	4% Positive

(C.B.)

Table VI is of interest as it shows that 4 per cent of a previously negative group were positive when retested three months after the primary test. The two toxins used were the same in both tests, controlled with heated toxins in the first test and uncontrolled in the second.

SUMMARY

1. Phenol (0.5 per cent) as a preservative in scarlet fever streptococcus toxin diluted for the Dick test has little or no deteriorating effect when the material is kept at ice box temperature.

2. Preservative plus temperature of 37° C. causes a fairly rapid deterioration.

3. A temperature of 37° C. alone causes no deterioration.

4. Purified toxin (salt and acetic acid method) seems to be more stable than unpurified toxin.

5. The injection of immunizing doses of toxin apparently induces a prompt resistance to infection when used in the presence of an epidemic of scarlet fever.

6. A very early modification of the skin test is produced in many individuals by such immunizations.

REFERENCES

[1]Jour. Am. Med. Assn., March 14, 1925, lxxxiv, No. 11.
[2]Proc. of Soc. for Exper. Bio. and Med., May, 1924, xxi, No. 8.
[3]Nesbit: Jour. Am. Med. Assn., March 14, 1925, lxxxiv, No. 11.

DISCUSSION

Dr. John A. Kolmer.—Dr. Murphy is to be complimented and thanked for summarizing his work on this interesting subject in so clear and instructive a manner. If 500 or more skin doses of toxin may produce a scarlet-fever-like reaction when given for the first dose, is it possible to immunize with a toxin-antitoxin mixture more safely? Has Dr. Murphy any experiments to show that dissociation occurs after absorption? I doubt if the method would be objectionable from the standpoint of serum sensitization if a method could be developed along the lines of the T-A mixtures for active immunization against diphtheria.

I should like to ask Dr. Murphy how the antitoxic and antibacterial serums compare in their influence upon the streptococcus complications of scarlet fever?

Has Dr. Murphy any data to contribute upon the results of Dick tests in scarlet fever convalescents?

Dr. Frank M. Huntoon.—Our work has been done with comparatively small doses for the reason that we were afraid. Other workers not limited by lack of courage and other considerations have used very much larger doses. You can produce a negative skin test in practically every individual showing positive if you give enough material but by doing

this you may produce a very severe reaction. You are going to limit its value as a prophylactic. On the other hand, the commercial houses would not dare to put out some of the doses employed. We had a letter from a doctor in Buffalo who insisted that we supply him with stronger material. In injecting five hundred students for prophylaxis, one student showed all the symptoms of scarlet fever; four or five students reported for the second dose and none came for the third dose. That is the answer to the question of large doses. Large doses must be worked up to gradually if the immunization is to last a great length of time. After even one injection you get a considerable number whose skin tests become negative immediately. Some become negative, then positive, then negative again. What is the explanation of this? The skin test dose is dependent on two different factors; the first is sensitiveness of the skin; second, the presence of antitoxin in the blood. If we assume that the first skin test dose desensitizes the skin and then further assume that after a certain period, three, four, or five weeks, or longer, antitoxin appears in the blood, then we have the explanation for this very peculiar effect. If it is true that by desensitizing these people you can render them resistant to scarlet fever, that is a very important point.

Dr. Herman Sharlit.—I would like to ask if any observations have been made with reference to these immunization tests; whether there has been any effect noted on the kidneys of the people tested.

Dr. E. Clarence Rice, Jr.—We have been doing some work in conjunction with the public health service on immunization. We have finally given it up inasmuch as the dose of toxin was evidently too small and complete immunity had not been conferred, although the parents of some of the children injected considered them as protected for life, a false sense of security. I see no reason for not producing immunity, provided a sufficiently large dose of toxin can be used. One thing we have noted is that nurses who have been on duty in the scarlet fever ward and give no history of scarlet fever showed a smaller percentage of positive Dick tests than those just entering the hospital for training. It seems quite likely that scarlet fever may occur without a rash. Dr. Dochez feels that the unconcentrated serum should be used in preference to the concentrated, inasmuch as some of the bactericidal properties are lost in concentration. It is my opinion that the advantages of the unconcentrated serum are more than offset by the increased likelihood of serum sickness.

Dr. H. A. Heise.—We have given many injections of scarlatinal toxin with no bad results. A neighboring doctor gave his children the first dose with no noticeable effect. The second dose also appeared to give no reaction, but several days later the child complained of poor vision. Two weeks after the injection the eyes were examined and reported as right 15/100 and left 20/100. A reexamination in two weeks revealed practically normal vision.

Dr. David H. Bergey.—I was very much interested in this paper. It seems to me that if we could find some way of checking up the immunity and the amount of antibodies circulating in the blood of persons giving a positive reaction we would help this matter very much. If we could take the persons with the negative reactions, try various amounts of their serum and see what amount of serum would neutralize its toxin, we would obtain useful information.

Dr. John A. Murphy (closing).—In reference to the effect on the kidneys of the scarlatinal toxin in immunization, with the small primary doses I have no personal knowledge of any kidney complications. We had a report from one physician who said that he had given 500 skin test doses and that the patient had considerable swelling of the face and neck, the puffy condition of nephritis but no examination of the urine was made. Another physician using 500 skin test dose as the first dose, reported three of the five patients showed a trace of albumin in twenty-four hours and two of the three cleared after forty-eight hours. This would indicate that large primary doses may cause a mild transitory nephritis. Reactions, either local or general have been noted mostly after the first dose and seldom after subsequent doses.

Those individuals that show a rather intense reaction to the Dick test are rather hard to immunize; it is difficult to eradicate the positive reaction. Such individuals very probably have a high degree of susceptibility and will require a large total number of skin test doses to result in a negative Dick test.

The eye condition reported here was probably due to a mild nephritis. Dr. Kolmer's question on the use of a toxin-antitoxin mixture for immunization for scarlet fever, I cannot answer other than to say that it would probably protect in some patients, and in some there might be disassociation of the toxin and the antitoxin. Adults are more likely to show marked reactions to injections of the toxin than children.

As to the merits of the administration of the antitoxin in treating scarlet fever, it undoubtedly has beneficial effect.

THE BIOCHEMIC AFFINITY OF VARIOUS METALS AND METALLOIDS FOR BACTERIA AND ORGANIC TISSUES*

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THE present study was undertaken with the view of determining primarily the combining power of cancer tissue and of certain bacteria with various metals and metalloids. It was hoped that, if specific affinities could be demonstrated, the resulting data might furnish us with "leads" that would serve as a starting point for chemotherapeutic investigations. This assumption was based upon the dictum of Ehrlich that "Corpora non agunt nisi fixata." If certain chemical compounds exhibited a special combining power with particular bacteria or cells, normal or pathologic, in vitro, one would be encouraged to determine whether the administration of such compounds in vivo would manifest similar affinities.

The results of our studies in this direction were, on the whole, disappointing in that they tended to indicate that the combining power of the substances investigated were general rather than specific. Nevertheless the information derived possesses certain elements of interest that may possibly be of value along other lines of research. This prompts us to publish them at this time. The method which we pursued was to allow the salts of various chemical elements in solution to come in contact under standardized conditions with organs or bacteria and later analyzing the organic matter for the element adsorbed. We were particularly interested in the affinities for cancer tissues and tubercele bacilli.

This problem has been attacked from other viewpoints by a large number of investigators. The influence of various elements on the tubercle bacilli has been reviewed by DeWitt[1] and by Wells, DeWitt and Long.[2]

In 1915 Coenen and Schuleman[3] injected "eosin selenium" and various fluorescent derivatives into tumor mice and found that the living tumor cells have no special affinity for these dyes. Weil[4] analyzed tumor rats after injections of sodium iodide and found that the blood contained the highest amount of iodine, then, in order, the tumor and the liver. His results indicated, too,

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ADSORPTION OF ELEMENTS FROM INORGANIC SALTS BY VARIOUS SUBSTANCES

NO.	COMPOUND USED	ELEMENT DETERMINED	MG. OF ELEMENT ADSORBED PER GM. DRY SUBSTANCE								ATOMIC WT. OF ELEMENT	PERIODIC GROUP OF THE ELEMENT	SERIES IN THE PERIODIC SYSTEM	APPROXIMATE PH
			TUBERCLE BACILLI	STAPHYLOCOCCI	NORMAL CALF'S BRAIN	SHEEP'S LIVER	SHEEP'S HEART	CANCER (LIVER)	CANCER (BREAST)	CANCER (LIVER)				
1	Auric Chloride	Gold	175	144	163	107	116	180		179	197.2	1	11	4
2	Mercuric Nitrate	Mercury	170	107	115	103	130	86		92	200.6	2	11	3
3	Pot. Tartro-Bismuthate	Bismuth	84	58	94	124	78			72	209.0	5	11	9
4	Uranium Nitrate	Uranium	80	130	77	91	70	89		84	238.2	6	12	6
5	Lead Nitrate	Lead	61	89	88	71	57	99		89	207.2	4	11	4
6	Silver Nitrate	Silver	41	102	67	39	31	52		60	107.9	1	7	6
7	Vanadium Dichloride	Vanadium	35						5		51.0	5	4	3
8	Cupric Sulphate	Copper	28	24	23	13	11		5	16	63.6	1	5	7
9	Ferrous Ammonuim Sulphate	Iron	21		23	8	8	6		9	55.8	8	4	7
10	Nickel Nitrate	Nickel	17						4		58.7	8	4	5
11	Sodium Tungstate	Tungsten	15								184.0	6	10	10
12	Potassium Iodide	Iodine	11								126.9	7	7	8
13	Sodium Tellurate	Tellurium	10						2		127.5	6	7	12
14	Arsenic Pentoxide	Arsenic	9			29	22	24			75.0	5	5	1
15	Thorium Chloride	Thorium	9						4	4	232.2	4	12	7
16	Sodium Selenite	Selenium	8		7				2	5	79.2	6	5	12
17	Barium Chloride	Barium	6		13	5	4	8			137.4	2	8	6
18	Calcium Chloride	Calcium	10						4		40.1	2	4	4
19	Zinc Chloride	Zinc	4		6				2	3	65.4	2	5	4
20	Magnesium Sulphate	Magnesium	4		10					6	24.3	2	3	7
21	Sodium Silicate	Silicon	4								28.1	4	3	14
22	Cesium Nitrate	Cesium	3								132.8	1	8	10

that diazo dyes produced an intense coloration in the necrotic areas of the tumors. The tissues were but slightly stained.

Cramer[5] exposed, in vitro, cells of a transplantable mouse carcinoma to the action of equimolar solutions of sodium chloride and calcium chloride. Cells treated with the latter showed a distinct inhibitory action. Sugiura, Noyes and Falk[6] investigated the growth of Flexner-Jobling rat carcinoma after grafts had been immersed in solutions of various salts at various hydrogen-ion concentrations. They found that Ca strongly inhibits growth and that a P_H of 6.0 appears more harmful to the carcinoma than a P_H of 8.0. Sugiura and Benedict[7] studied the effect of 32 different salts, administered orally, on the growth of Flexner-Jobling rat carcinoma. Copper sulphate, arsenious oxide, potassium carbonate and calcium chloride showed a perceptible, though not marked, retarding influence upon the growth of the tumor. Copper sulphate was the most effective in this respect and appeared to have some immunizing action against the tumor. Analyses made by Robin[8] indicate that cancerous tissue fixes silica, lime and to a lesser degree magnesium.

Our work consisted in exposing the bacilli or finely ground normal or cancer tissue to the action of solutions of various salts, removing the excess of solution, washing and analyzing the residue for the adsorbed element that could not be removed by repeated washing. The results are presented in the table.

DISCUSSION

Eight series of experiments were carried out with tubercle bacilli, staphylococci, cancer of human breast, cancer of human liver (two experiments), sheep's heart, sheep's liver, and calf's brain.

Examination of the above table discloses the fact that gold and mercury lead the list in combining power with bacteria and tissues, with bismuth, uranium and lead following in sequential order.

We were at first rather encouraged that gold and mercury exhibited the greatest affinity (among the elements tried) for tubercle bacilli, as these two metals in one form or another have been long used in tuberculosis and are believed by some to exhibit an influence upon the disease. The suggestive value of the findings was, however, largely nullified when we discovered that these two metals also exhibit a high degree of affinity for the staphylococcus and for various organic tissues.

It will be noted that all of the substances at the top of the list have high atomic weights, but on the other hand thorium and tungsten, which have likewise high atomic weights, exhibit a low combining power. The substances at the head of the list fall in different "periodic groups of elements," so that the periodic grouping exhibits no influence upon the behavior in relation to proteins.

It is rather interesting to note, however, that four of the five elements that show the greatest combining power with organic material are in the eleventh series in the periodic system, and the fifth is in the twelfth. Whether or not this observation possesses any significance, further study alone can determine.

That the method employed by us gives reasonably accurate results is suggested by a comparative study of the experiments with two different specimens of cancer of the liver made at different times. In the first test 180 mg. of gold

was recovered and in the second 179 mg. The figures for the other elements, while a little wider apart, nevertheless indicate that the technic used is thoroughly reliable.

METHODS

The tubercle bacilli (human and bovine mixed) were washed free of glycerine, dried between filter paper, a ten-gram sample removed for drying to constant weight at 105° C., and the remainder weighed in five-gram lots into 50 c.c. centrifuge tubes. To each were then added 20 c.c. of a solution of such concentration that it contained 2 per cent of the element to be determined. The tubes were stoppered, thoroughly shaken, heated at 37° C. for three hours, and placed in the ice box overnight. The mixtures were then centrifuged and the supernatent liquid discarded. About 30 c.c. distilled water were added, and the whole mixed, centrifugalized, and the supernatant liquid discarded. This process was repeated seven more times except that, before discarding the sixth washing, tests were made to determine whether the wash water still contained any of the element to be determined. It was found that the sixth washing was generally negative. The bacilli were washed twice more, however, drained as completely as possible, and transferred quantitatively to various containers to be analyzed for the adsorbed elements.

The staphylococci were obtained dry. Two-gram lots were used and treated as above.

All the tissues were passed through a small meat chopper; ten grams were removed for the moisture determination; the remainder was weighed into centrifuge tubes, ten grams in each, and treated as above. All results were calculated to a dry basis.

For the determination of mercury,[9] bismuth, lead, copper, iron, nickel and tungsten, the organic matter was destroyed with concentrated sulphuric acid and potassium sulphate in the ordinary Kjeldahl manner. Mercury and bismuth were weighed as the sulphides; lead as the sulphate; copper precipitated as sulphide and ignited to the oxide; iron and tungsten as the oxides; and nickel as the dimethylglyoxime.

For the determination of gold, uranium, vanadium, tellurium, selenium, thorium, magnesium, zinc, and silicon, the organic matter was destroyed with aqua regia. Gold, selenium and tellurium were weighed as the metals; uranium, vanadium, thorium, magnesium and silicon as the oxides; and zinc as the pyrophosphate.

For the determination of silver, barium, calcium and cesium, the organic matter was destroyed by ashing. For the silver estimation the ash was leached with concentrated nitric acid; for barium and calcium, with dilute hydrochloric acid; and for cesium, with dilute sulphuric acid. Silver was weighed as the chloride; and barium and cesium, as the sulphates. Calcium was estimated by titrating the oxalate with standard permanganate.

For the arsenic the organic matter was destroyed on the water-bath with hydrochloric acid and potassium chlorate; the arsenic precipitated as the sulphide, dissolved, reprecipitated with magnesia mixture, and weighed as magnesium pyroarsenate.

Iodine was determined by Kendall's[10] method, slightly modified to take care of the larger amounts of iodine.

CONCLUSIONS

We have not been able to demonstrate, in vitro, any specific affinity of metallic and metalloid elements for organic tissues or for germs. Although no selectivity could be demonstrated, elements known to be therapeutically active (excepting arsenic) are adsorbed by tissues to a marked degree.

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THE UNIVERSITY OF MARYLAND CHRONOGRAPH, ITS CONSTRUCTION, ADVANTAGES, AND APPLICATION TO THE NEEDS OF THE PHYSIOLOGIC AND PHARMACOLOGIC LABORATORIES*

By O. G. Harne, Baltimore, Maryland

An instrument to record time for experimental physiology and pharmacology has commanded considerable attention, especially in the last three decades. Many have been produced and used with remarkable success. However, few, if any, have been satisfactory, except for individual work. All, it is true, have functioned in their own way and have facilitated experimentation beyond measure, but as time passes, needs arise which they are unable to satisfy. Modern laboratories no longer require one rate of time only, but time intervals of varied lengths. Then it is, that special and more complicated devices have to be originated and used, which will (by shunting in other units) enable one to use the time interval best suited to the needs of the experiment.

One of the simplest recorders of the more accurate type is shown in Fig. 1. The metronome type is driven by a spring. The pendulum so activated is made to operate a horizontal arm to which are fastened contact-points (one on each end) which alternately dip into a mercury bath, making the contacts. From these mercury-cups the wires lead to the recording instrument. Many of these simple types are still in use. Where simple contacts are required, within the range of this instrument, and where it can be used for individual work, it surpasses the metal contact instruments. It has, however, the disadvantages of any mercury contact instrument in that it gives an arc and the mercury needs to be renewed.

Fig. 2 shows an advance in the metronome time recorder. It has all the advantages in range that Fig. 1 has and is activated electrically, doing away with the variations sure to occur when a spring is used. The swing of the pendulum can be broken off at pleasure. This is an advance from the mechanical point of view.

The pendulum type of recorder seemed to be preferred to any other for a long time. The accuracy with which the contacts were made was doubtlessly the cause, and because these instruments could be purchased in different forms. (It was really applying an instrument, which was built for delivering time intervals for another purpose, to the needs of experimental physiologic and pharmacologic laboratories.) This in part explains why chronographs, time recorders, etc., for use in physiologic and pharmacologic laboratories have not advanced beyond the place they hold today. The need, it is true, is rapidly increasing, and necessity for accuracy is keeping pace with it. So, we look for-

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ward to the development of the ideas which mark a new departure from the old conservative system.

We have seen that the metronome type of recorder has many advantages in accuracy, and disadvantages in the limited range, exposed mercury cups, and in some cases a spring tension for activating the pendulum, which is pivoted at the bottom.

From this departure we come to the clock type. In appearance these simpler forms have much the semblance of the common wall or grandfather's

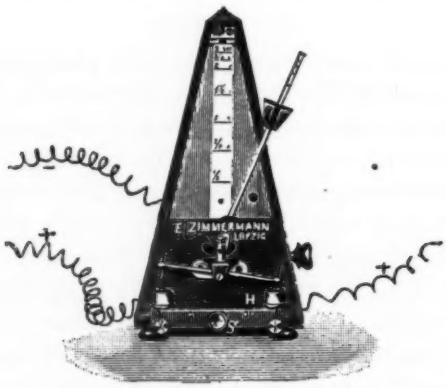

Fig. 1.—Metronome type, with exposed mercury cups, spring driven. Range from ½ to 3 seconds.

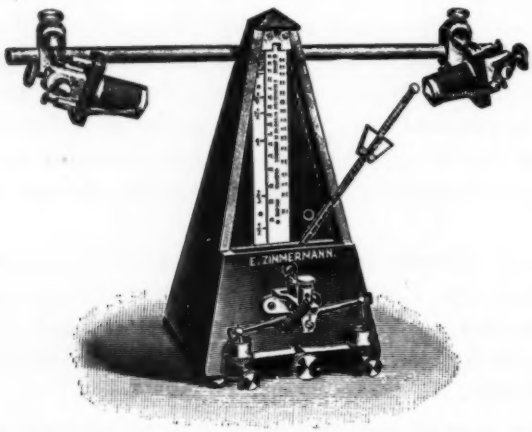

Fig. 2.—Metronome type (electrical) having exposed mercury cups. Range from ½ to 3 seconds. Electrically propelled.

clock. There are numerous models, but here we can refer briefly to some of the representative types only.

Fig. 3 represents well one of the more simple types which consists for the most part of a pendulum activated by a weight which pulls on the drive shaft, furnishing power to the ratchet which, through a pawl, activates the pendulum, the swinging of which tilts an arm. This arm is pivoted in the middle, and is connected to the pawl. The contacts are made similar to those of the metronome shown in Fig. 1.

Fig. 4 is the contact clock according to Bowditch-Baltzar. Through the

swinging of a carefully adjusted "second" pendulum the contact point is moved forward one notch, and by means of a moving strip of metal the current is broken. The contact points are pegs of metal, arranged in concentric circles, accurately spaced so as to record intervals of 1, 2, 3, 4, 5, 10, 15, 20, 30, and 60 seconds respectively. By making adjustment, the contact points can be made to so respond that the *break* stimulus can be given instead of the *make*. The workmanship on the rachet and pawl is such that the interval of time is very accurate, yet the mechanism of the clock is very simple. It will be noticed that there is still a close adherence to the pendulum type of recorder all the way through this series. Of still greater importance are the following combinations of the pendulum and electromagnet types. In these clocks the weight and spring are not used, yet the pendulum is retained.

Fig. 5 is a cut of the late Prof. Brodie's pendulum time-marking clock.[1] This clock consists of a half-second pendulum, the swing of which is maintained

Fig. 3.—Pendulum type, with mercury cups. Weight, ratchet, and pawl activation.

Fig. 4.—Contact clock (Bowditch—Baltzar). Pendulum type activated by weight. Contact points are metal.

electromagnetically. The pendulum acts by means of a pawl upon a ratchet wheel, which has sixty teeth, and is moved forward one notch (tooth) for each double swing of the pendulum. As it moves, one of the teeth at the side of the wheel is made to depress a spring, and thus close the circuit of the writing signal. This gives a sharp tracing by the chronograph. On the same axis are other ratchet wheels, each having a different number of teeth, depending upon the time interval they are to give. In the clock as reported, there were wheels having 30, 6, 2, and 1, respectively. This would mean that the contacts could be made at intervals of one, two, ten, thirty seconds and minutes.

Fig. 6 shows the Harvard electric clock. This was reported by W. T. Porter,[2] and would come in this class. This is a portable type and is of the same general design. So far as the time interval is concerned, it delivers the same rates as does Fig. 5. In action, a pendulum moves a pawl, which each

second turns a wheel one-sixtieth of its circumference, making a contact which activates a magnet to keep the pendulum "Normal." On the rim of the wheel are contacts, which cause a magnet (signal) to record each one, two, ten, thirty, and sixty seconds as does the one illustrated in Fig. 5. The driving circuit is independent of the time circuit. The signal contact spring can easily be moved along the transverse bar, bringing it in contact with the wheel delivering the time interval desired. A leveling device is provided, which is necessary when one hopes to use it as a portable instrument. Leveling of this instrument is facilitated by the addition of a point on the base over which the pendulum is carefully balanced when at rest.

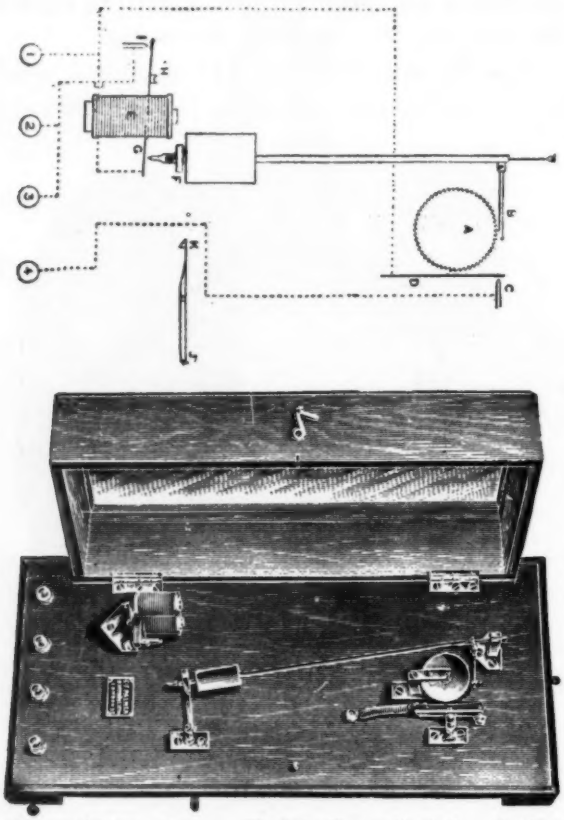

Fig. 5.—Pendulum time-marking clock.[1] Electrically activated. Contact points are metal. (T. G. Brodie.)

Through this entire series of pendulum type clocks we have observed several striking contrasts, both in application of the driving mechanism, and in the manner of making the contact. A combination of many of these (except the mercury-cup principle) with many refinements, will be found in the astronomical clock after Clemens Riefler,[3] Munich, Germany. (One of these clocks will be found in use at The Bureau of Standards, Washington, D. C.) It is claimed to be the most accurate clock in the world, the probable error lying somewhere between 0.01 to 0.02 of a second in twenty-four hours.

Some idea of its accuracy can be obtained from some illustration which brings to mind the linear error on a very fast drum. This of course will vary

according to the lag in the chronometer; the resistance in the lead-in wires, and several other factors (making difficult a very accurate measurement). Then we shall profit more, in our attempt to illustrate its accuracy, by disregarding these extrinsic factors, and deal with the clock itself.

If we were to picture a six-inch Harvard, "slow and fast combination" kymograph, revolving at a rate of once per second, and the recording instrument executing the contact made within the clock, without lag, the linear error would be slightly less than one-tenth micron. Another illustration might be

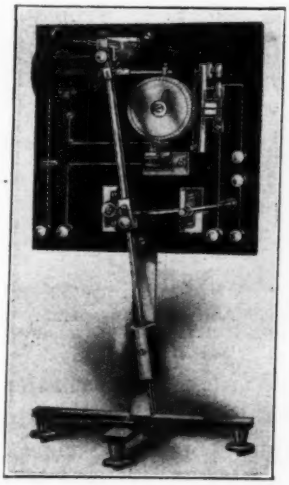

Fig. 6.—Harvard electric clock. Portable pendulum type. Electrically activated. Adjustable. (W. D. Porter.)

Fig. 6-A.—Astronomical clock. (Riefler.)

used. If the error recorded were superimposed upon an accurate scale, a high-power microscope would be necessary to detect it. By careful installation and proper adjustment it is possible to maintain this accuracy, constantly.

It is enclosed in an air-tight glass chamber to which are connected means for partially exhausting the air, thus changing the pressure within the clock to any desired amount. After the proper adjustments are made, pertaining to pressure, the clock requires little attention.

It is wound every half minute by a battery of dry cells (4.5 volts) the average life of which, for this purpose, is six months.

The pendulum is supported on agate planes, and operates a wheel having sixty teeth, one of which may be removed to indicate the beginning of the minute. A ruby jewel, bearing upon this wheel, operates an electric contact each second as the jewel passes over the teeth. This contact opens the circuit of a relay, which distributes the signals to the various laboratories, for operating chronographs.

It will be seen that the pendulum type of recorder is used in this most accurate instrument, and that the spring, the source of power, is kept at a constant tension by frequent windings. The effects of atmospheric changes are reduced to a minimum by the glass encasement, as are convection currents.

Further information may be obtained, pertaining to the construction and cuts, if reference be made to D.R.P. No. 100870, München, 1903.

A photographic reproduction is shown in Fig. 6-*A*.

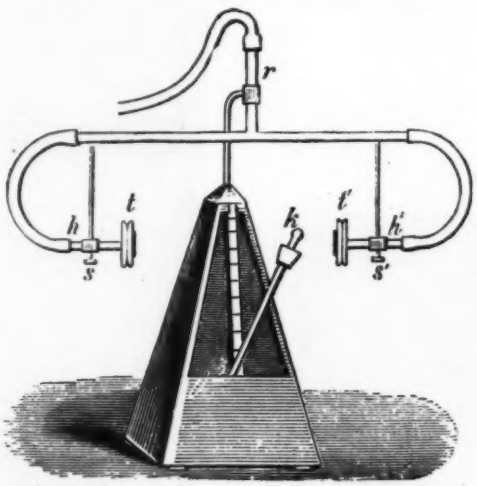

Fig. 7.—Transmission chronograph. Metronome type. Activated by spring. Impulse is transmitted through a closed system to recording instrument. (Klemensiewicz.)

Before leaving the pendulum type of recorder, we must refer to the transmission chronograph (Klemensiewicz). This is an air transmitting instrument, consisting of a metronome to which at its upper portion is connected a *C*-shaped pipe in such a way that the swinging of the pendulum strikes against tambours, one on each end of the curved pipe. By each movement of the pendulum the tambour is struck, causing a depression in the diaphragm. This in turn is transmitted through the closed system to the recording tambour. It will be noticed in Fig. 7, at the point *r* a tube (rubber) is connected which transmits the impulse to the recording apparatus. The pendulum is activated by a spring.

Departing from the pendulum type of recorder we come upon a class which marks a distinct innovation in time-recording apparatus. They are small and portable and more especially designed for individual work. Accuracy will be found in this class of instrument equal to that of the pendulum type. One of the simplest of this class will be found illustrated in Fig. 8. This recorder

was described by C. C. Lieb.[4] It consists of an Ingersoll watch to which is attached, instead of the second hand, a toothed, geared wheel which activates a writing lever. Time may be recorded in minutes alone; in minutes and seconds, or in minutes and five second intervals. The writing lever is made of straw; the writing point of celluloid.

Without doubt the most accurate and complete, small, portable chronometer on the market today will be found in the Jaquet graphic chronometer. It consists of an accurate clockwork with anchor escapement; two dials on one side, one of which totals seconds, and the other, the minutes of operation. A small lever placed beneath the box will set the instrument at *o* instantly. It is also arranged to give an electric signal through a magnet, being provided with binding posts on the front side. This contact for signaling the beginning

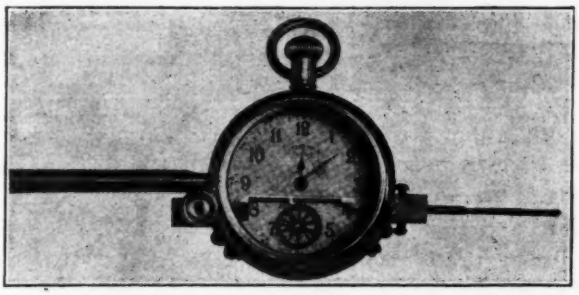

Fig. 8.—Time marker (Lieb), consisting of an Ingersoll watch to which is attached, instead of a second hand, a tooth-geared wheel. This wheel turns 1/60 of a complete revolution at each escapement of the anchor, activating the writing-point. It is adjustable to deliver different rates of time.

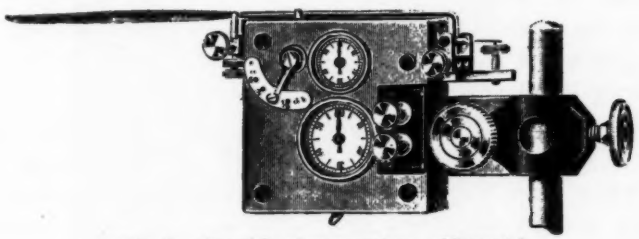

Fig. 9.—Graphic chronometer. (Jaquet.)

of the observation, is made with the lever which establishes the zero reading. It can also be made to record time at a distance. It will write in a vertical or horizontal position. It appears on the market in two executions, and is capable of delivering one-fifth, one, three, and six seconds. This class of instrument is the only type taking advantage of the anchor escapement principle. An illustration of this chronometer is given in Fig. 9.

Having considered well the advantages and disadvantages of all the different types of chronographs and time markers, and having worked with many of them personally, it appeared to me that instead of having individual time markers, which, by the way, are subjected to use by inexperienced workers principally (that is for student's laboratory work) and in a way are very satisfactory, but are apt to be out of order when most needed, it would be a remarkable advance, so far as handling a student's laboratory is concerned, to have a central clock, capable of taking care of the entire laboratory, delivering

to any or all tables therein the time interval most suited to the needs of each individual experiment. This led to the summing up of the needs of the average modern laboratory. In this summing up it was found that there were needs for the following: time in one second; seconds and minutes together (to facilitate counting time, and analyzing records) and then an increased rate to compare with the tuning fork of about one one-hundredth, or at least one-tenth of a second. This gives a range taking care of average experimentation, and the fast rate for measuring the phases of individual tracings on a fast drum. This refers to records of time. Then there is a great need for the automatic stimulating machine; something that will give stimulation through the inductorium at a constant rate and intensity; one that can be varied to suit the needs of the experiment without altering the time record. All this necessitates a somewhat complicated mechanism, simple to operate, and positive in execution. In fact, the percentage of error present with either without the other is so high that even the inexperienced experimenter will ask for explanations.

With these things in mind the University of Maryland chronograph[5] was produced. It has been in use in our laboratory, and without an exception has filled all the needs of our students' laboratory. Not only is it accurate in the rate of stimulation, and time recording, but constant in intensity, and simple to operate. An illustration of recording time in seconds and minutes, and at the same time stimulating the gastrocnemius muscle of a frog, is given in Fig. 10. It will be seen that the rate of stimulation was started at about one in three seconds, then slowly increased until it was being stimulated approximately twice per second. At about the center of the record as indicated thereon, the rate was slowly decreased until the original rate was established. The kymograph paper upon which this record was made is not the glazed surface variety, but regular Drug Bond wrapping paper. A celluloid point was used on the recording lever. At the time this record was made six other tables were in use. Table one was using minutes only; table two was using seconds and minutes and tables three, four, five, and six were using one-fifth of a second. This shows the possibility of any one table having at its service the time interval best suited to its needs. There will be a record shown illustrating the different rates possible at any one time.

Fig. 11 is shown to give some idea of the kind of record made when standard apparatus is used. The paper upon which this record was made is Drug Bond wrapping paper. The kymograph was a standard Harvard, which is driven by a spring. It will be seen that for fast records the rate is not constant, but in this record where the character of the signal is to be illustrated that does not interfere. The rates as indicated on the record, therefore, can be given only approximately, so far as the speed of the drum is concerned. The contact is made and maintained for one one-hundredth of a second. This throws the *make* and *break* so close together that even in a fast drum record, such as can be obtained with the Harvard "slow and fast combination" kymograph, it is not possible to distinguish the break. In many kinds of experimental work this is an advantage. It will be noticed in this record that all contacts are sharply registered on the record. The "minute" can be recorded

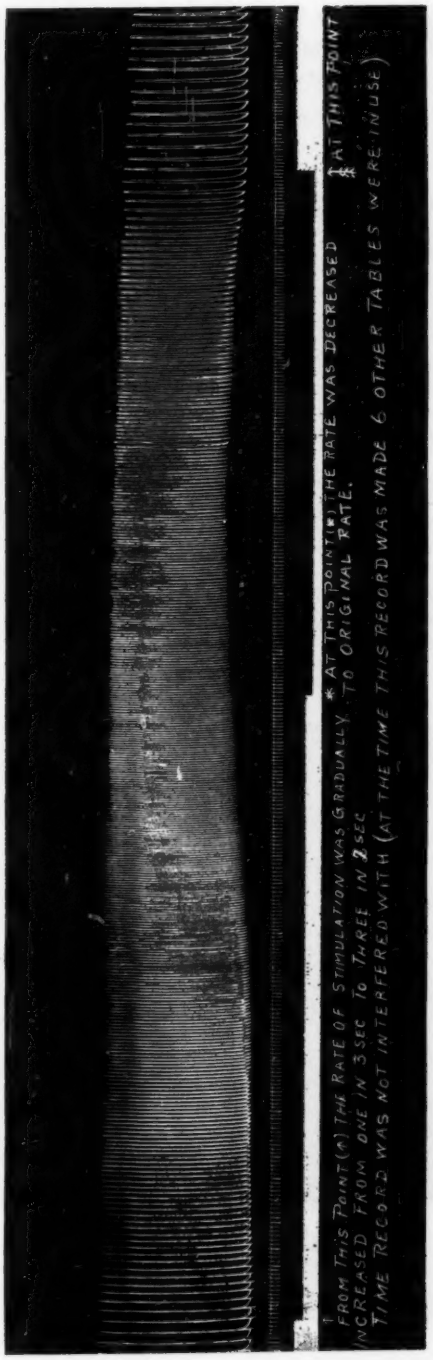
FROM THIS POINT (↑) THE RATE OF STIMULATION WAS GRADUALLY
INCREASED FROM ONE IN 3 SEC TO THREE IN 2 SEC
TIME RECORD WAS NOT INTERFERED WITH (AT THE TIME THIS RECORD WAS MADE 6 OTHER TABLES WERE IN USE)
* AT THIS POINT (↑) THE RATE WAS DECREASED TO ORIGINAL RATE.
↑ AT THIS POINT

Fig. 10.—The broad band below the record at the first arrow (↑) shows the point at which the rate of stimulation was gradually increased from one in three seconds to three in two seconds. The asterisk (*) indicates the point at which the rate was gradually decreased to the original rate. The time record was not interfered with, nor were the instruments being used on the other six tables.

either by a break in the line, of a second duration, or by a sharp single line, depending upon the adjustment of the timer. These different methods of recording minutes will be found on the record as shown in Fig. 12.

Fig. 12 is a record showing the different time intervals that can be given at one time to any or all of twenty tables. The number "twenty" is mentioned because of the instrument being described, it having been made to care for a laboratory of twenty tables. It can be made by the insertion of a dif-

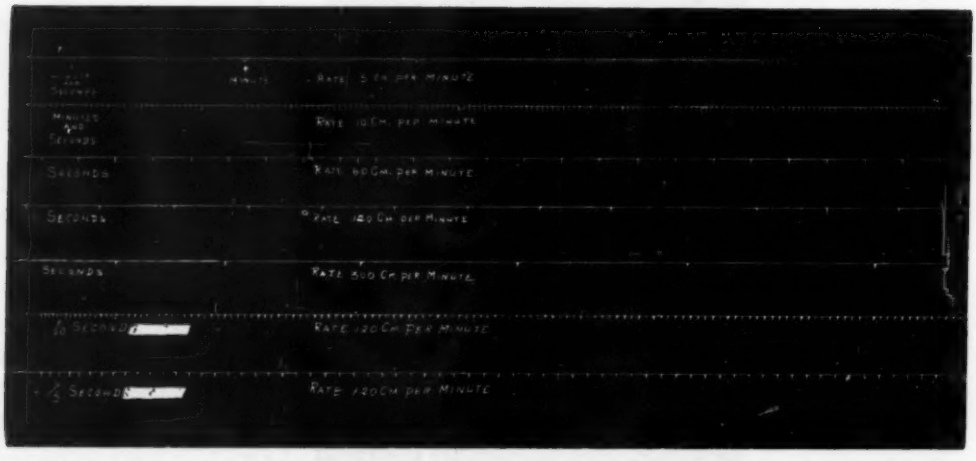

Fig. 11.—Showing how different time records will appear when kymograph is revolving at different rates. Also how seconds and minutes are recorded with one magnet.

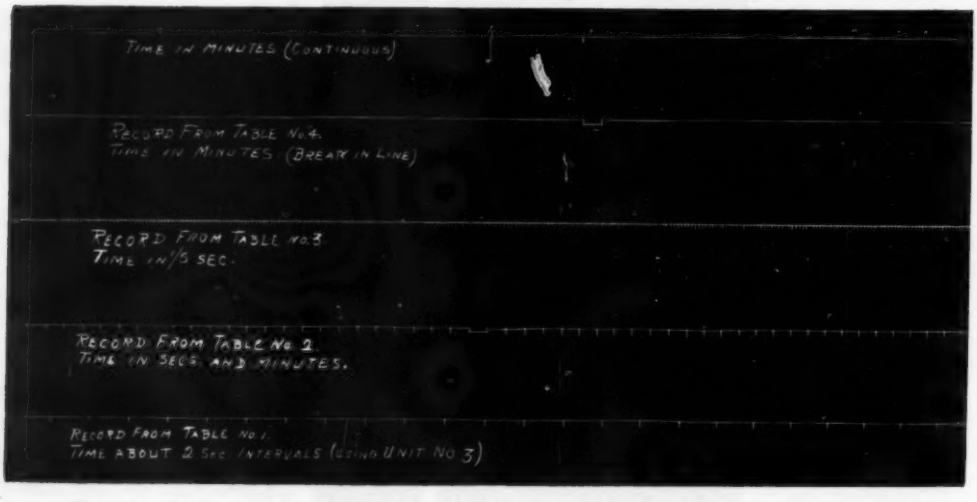
Time in Minutes (Continuous)
Record From Table No. 4.
Time in Minutes (Break in Line)
Record From Table No. 3.
Time in 1/5 Sec.
Record From Table No. 2.
Time in Secs. and Minutes.
Record From Table No. 1.
Time About 2 Sec. Intervals (Using Unit No. 3)

Fig. 12.—The top line shows the record made when minutes are being recorded and the timer is set to make a sharp contact. The second line illustrates how minutes can be recorded on a very fast drum when the timer is set to cause a break in the line lasting one second. The third line shows ⅕ second as recorded with a standard Harvard Magnet. The fourth line shows minutes and seconds on a fast drum. The bottom line is shown to illustrate the use of unit No. 3 for giving time at intermediate rates.

ferent commutator, to take care of even one hundred or more. That is, it can be made to care for the needs of the number of tables mentioned without having any two tables operate from the same line at the same time. The first record at the top of Fig. 12 shows how minutes may be recorded on a very

fast drum when the commutator is adjusted to deliver a sharp contact. The second record (Fig. 12) shows the commutator adjusted to give a contact of one second duration. The third shows how one-fifth of a second will be recorded, and the fourth, seconds and minutes, with the commutator of the minutes unit set to make a break in the line, lasting for exactly one second. It should be mentioned here that while the magnet is being held down, during that second, recording minutes, no other table is being interfered with. The instrument is so made and adjusted that an entirely independent current is operating the minute signal, while the second signals are continuing on their own circuit. The fifth record (bottom) shows the possibilities of shunting into the time recording service, the unit for stimulation which makes it possible to get time in either half seconds or two seconds, or a fraction thereof. The record here shown is time at a rate of approximately two second intervals. This is adjustable, and is the one unit that can be fitted to the needs of special work. This illustrates the use of the chronograph, so far as the time recording possibilities are concerned, showing how the entire instrument can be applied

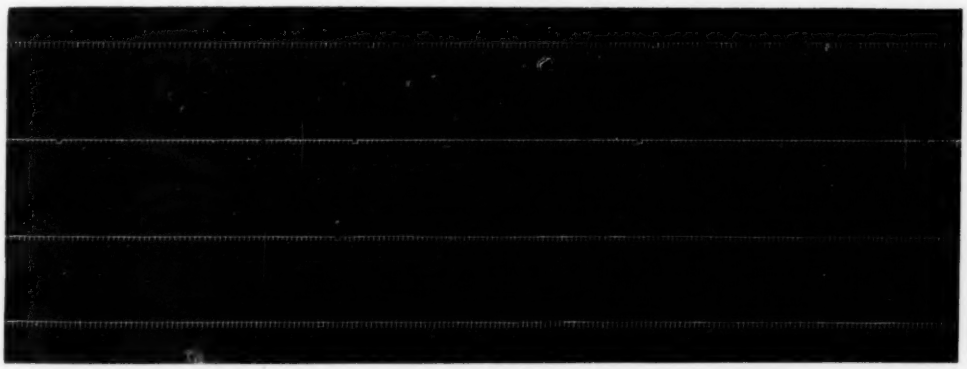

Fig. 13.—Showing how the chronograph records the different time intervals when all magnets are started on a vertical plane. This record was made by connecting four tables together in such a way that their magnets would record on one drum.

to the recording of time or to both time and stimulation, as the experiment requires. The records as given in Fig. 12 were made simultaneously, the magnets being connected to leads from different tables as indicated on the record.

Fig. 13 illustrates how the records would appear if the tables were all writing upon one drum simultaneously, provided the points of the magnets were all started upon a vertical line. It will be seen that the record at the bottom records minutes first; three seconds later, the second magnet records, and three seconds later, the third and so on. By the time the last of the twenty tables records minutes the first will be within three seconds of recording minutes again. No two tables record minutes simultaneously. This allows some little time for the chemical reaction in the battery to take place and insures positive execution.

It will be seen from the records shown that there is not a trace of interference, one table with the other. While all tables receive their current at a definite rate, at no time are there two receiving impulses from the chronograph. This involves a perfectly timed commutator, a compound circuit, and

a constant "input." These important parts are believed to be well represented in this instrument.

Fig. 14 is a perspective of the University of Maryland chronograph. The rheostat (*R*) is a Beck Brothers 750 Ohm 0.6 Amp., and governs the input to the motor (*M'*) which is a Hamilton Beach Home type, rated ¼ h.p. *AC* or *DC*, 3000 r.p.m., and drives the machinery through a spiral gear *S'*. This

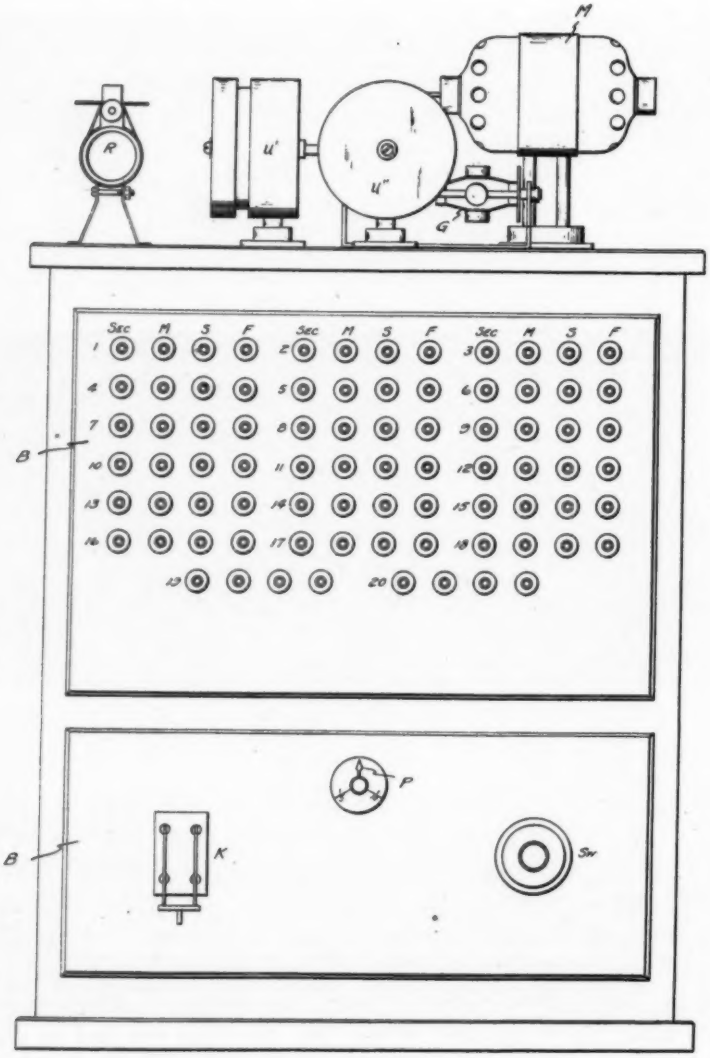

Fig. 14.—*R*, Rheostat; *U'*, unit for stimulation; *U"*, unit for minutes; *G*, governor; *M'*, motor; *B*, main switchboard; *1-20*, inclusive groups of switches representing laboratory tables; *Sec.* Switch to U^3, Fig. 15; *M*, switch to *U"*; *S*, switch to *U'*; *F*, switch to indicator *P*; *B'*, small switchboard; *K*, laboratory input switch; *P*, indicator for fast time; *Sw*, 110 volt switch.

worm drive engages with a 32 pitch (diametral) spur gear (*S*) having 80 teeth (Fig. 15). This gear is fastened to the driving shaft, "pressed on," and becomes the driving shaft proper, common to all units (*D*) (Fig. 15).

In Fig. 14 *U'* is the unit wherein the contacts are made which give stimu-

lation at intervals varying from half seconds to three seconds; *U″* the unit which makes the contacts for minutes. There is another unit, which is not shown in this perspective, lying between *U″* and the motor. This unit is the "second unit" and can be seen in Fig. 15.

The governor (*G*) is a "three ball breaking type," and engages with the drive shaft (*D*) through a worm and spur gear (*w*) (Fig. 15). The efficiency of this device is marvelous.

The switchboard *B* (Fig. 14) is fitted in, and becomes the upper panel of the front of the cabinet. On it are mounted the switches in groups of four, twenty in number, making a total of eighty. The small numbers appearing before and on the left side of each group of four switches, represent the num-

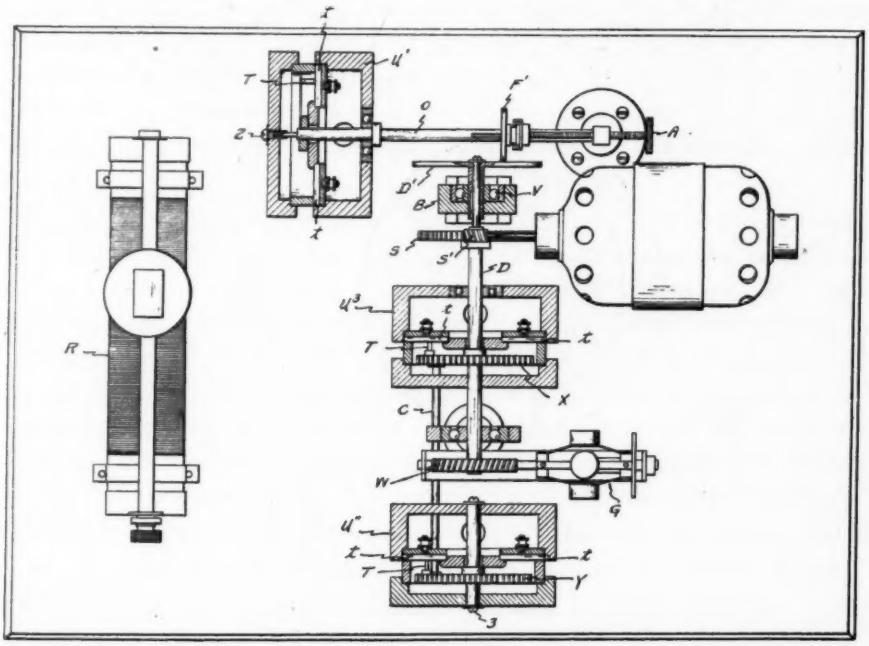

Fig. 15.—*R*, Rheostat; *U′*, unit for stimulation; *U″*, unit for minutes; *U³*, unit for seconds; *2*, ground-wire to unit *U′*; *T*, brush; *t*, contact; *O*, drive shaft; *F′*, friction wheel; *D′*, friction disc; *A*, screw set; *B*, thrust bearing unit; *V*, new departure ball-bearing No. 1200; *S*, spur gear 80 cogs; *S′*, spiral gear; *D*, propeller shaft; *X*, gear wheel (one cog); *Y*, gear wheel 80 cogs; *W*, spur gear to governor; *G*, governor; *3*, ground-wire (common to unite *U″* and *U³*).

bers of the tables to which the wires of that particular group run. The lettering over the vertical rows of switches represents the execution. For example, *Sec.* for seconds, *M*, for minutes, *S*, for stimulation and *F* for fast time. In order to have fast time at either one-tenth or one-fifth of a second it is necessary to turn indicator *P* (Fig. 14) to the point desired, and then pull switch marked *F*. If fast time is not desired, it is not necessary to readjust the indicator *P* but simply use switch *F*.

Another switchboard *B′* forming the lower panel in the front of the cabinet, contains the double knife switch *K* which connects the laboratory tables to the instrument (chronograph). It is in the circuit on the "ground" side. Indicator *P* is mounted also on this panel, the explanation of which was

given in the preceding paragraph. The diagram *P* (Fig. 20) explains the wiring. The core of this indicator is an insulated brass-bakelite commutator which when indicator *P* (Fig. 14) is turned to one-fifth of a second serves as a common conductor for five of the twenty contacts, and is shunted through switch *F* (Fig. 14) to the table desired. The same principle applies to the

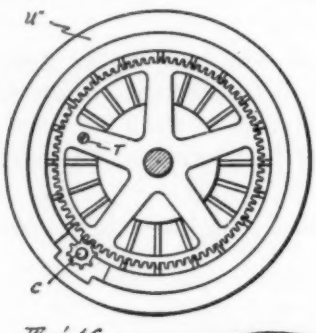

Fig. 16

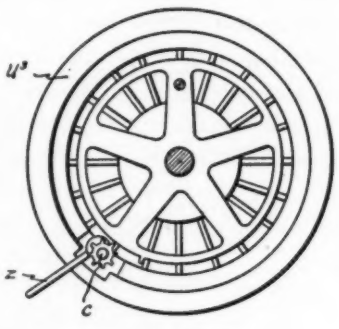

Fig. 17

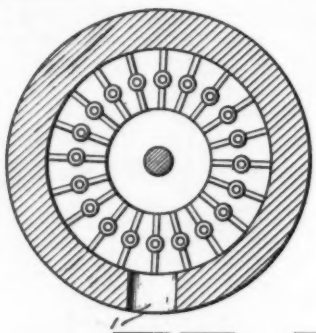

Fig. 18

Fig. 19

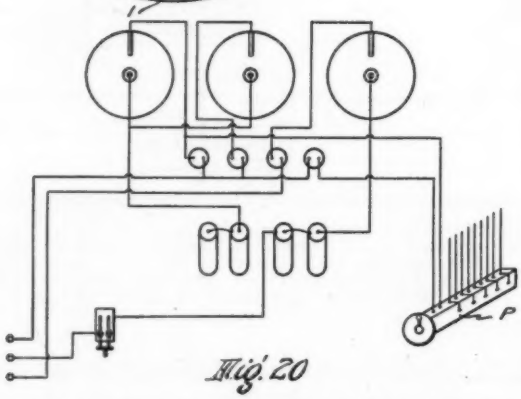

Fig. 20

Fig. 16.—*U"*, case of unit for minute contacts; *T*, brush; *C*, cam shaft.
Fig. 17.—*U³*, case of unit for second contact; *Z*, timer; *C*, cam shaft.
Fig. 18.—Reverse side of units *U"*, *U'*, and *U³*; *1*, hole for support.
Fig. 19.—*T*, contact (actual size).
Fig. 20.—Wiring diagram showing all three units; one group of switches; four dry cells (Red Seal); laboratory input switch; three binding posts (on laboratory tables); fast time unit (*P*).

one-tenth of a second except that in this case ten of the contacts find the commutator a common conductor. It will be seen here, that there can be no division of these two time intervals. The entire mechanism is either delivering all one-fifth or all one-tenth. It is easily rearranged, in that the core is responsible

for this execution. The driving switch 110 V. *Sw* completes the trio of mountings. Through this switch the AC or DC current is supplied to the motor through the rheostat (*R*).

Fig. 15 is a cross section of the contact units, bearings, friction transmission, and gearing. The unit, *U′* is responsible for stimulation, the rate of which is governed by the position of the fiber wheel *F′*. This wheel is adjusted to suit the needs, by use of the "screw set" *A*. Having been set at the point best suited for the experiment, the disc *D*, by means of another "screw set" not shown on this cut, but at the base of, and under the bearing unit *B*, is brought into contact with fiber wheel *F′*. This starts the propeller shaft *O*, to which is fastened the contact brush *T*, (this brush is made of 50-50 half hard brass) causing contacts to be made on each of twenty inlaid-contacts *t*, also *t* (Fig. 19). This unit can be thrown out of service at will. It will be noticed that the disc, and its thrust unit is firmly connected to the bearing *V*, and that the entire unit slides over a key in the propeller shaft *D*. This keeps the wheels at right angles to each other and the disc in a vertical position at all times.

The unit, U^3, gives the contacts for seconds. Its construction is similar to that of *U′* except that the brush (*T*) is attached to the gear wheel (*X*). Unit *U″* delivers minutes and is driven through a cam shaft from unit U^3. In each complete revolution of the wheel *X* of unit U^3 a cog engages with the cam turning the gear wheel *Y* of unit *U″* one-eightieth of its circumference. This causes the brush to rest for one second on each contact as it revolves, providing the timer *Z* (Fig. 17) is so adjusted. It can be made to rest on either side of the contact. In this case it passes over without interruption and makes the contact in one one-hundredth of a second. This gives a sharp signal by the chronometer. Otherwise it gives a break in the line of one second duration.

Fig. 16 illustrates the relation of the cam (*C*) to the contact surface, the position of the brush, the actual size of the unit, and the "open fan arrangement" of the contacts. This is common to all units.

Fig. 17 gives the inside appearance of the unit U^3 showing the timer, cam, and brush.

Fig. 18 gives the reverse side of the contact unit, showing how the connections are made to the contacts, also the hole into which the unit supports are inserted, *1*. These contacts should be soldered to avoid loose connections. The support is a hollow pipe through which the leads disappear to the cabinet and switchboard.

Fig. 19 shows the contact inset. (Actual size.)

Fig. 20 is the wiring diagram, showing the arrangement of the double circuit, and its connection to the different units. It will be seen that the ground wire is common to all tables in the laboratory and that the switch is in the circuit on the "ground side." Referring to the ground wire, and its connection to the chronograph, it will be found connected with the central screw of both units *U′* and *U″* marked *2* and *3* (Fig. 15).

SUMMARY

In the production of The University of Maryland chronograph, the outstanding features of time markers, chronometers, and chronographs, were considered. While their principles were not adopted, the results obtained with them were. The ideal chronograph ought to serve an entire laboratory, in the same way any of the best portable types serve the individual table. This was the goal set, and in a large measure accomplished. Having used the new chronograph in our laboratory of twenty tables, and without an exception, with excellent results, it is only right that it should be used unselfishly for the advancement of laboratory work in other laboratories. With it, it is possible to supply current to any or all of twenty tables, or more if desired, in the form of single induction shocks without interfering with any other circuit. It will supply to one table, one time; to another, a different rate; to still another, both time and stimulation if desired. In other words, no single table in the laboratory is at the mercy of any other. It is capable of doing three independent pieces of work for any one or all tables. It will record time in seconds and minutes, and deliver single induction shocks at a constant rate to the inductorium, at a rate varying from three per second to one in three seconds. This rate can be changed to suit the laboratory requirements, if this rate is not within the range of the needs, with very little trouble, and a few cents expense, or can be designed to fill the requirements without extra expense. It is so constructed, that the units can be fitted with replacement parts, in case a laboratory expands or diminishes. This makes it possible to keep it up to the minute. It can easily be disassembled, only one part being connected rigidly with the propeller shaft. It is positive in execution, adjustable, and foolproof; simple to operate, and durable.

I am indebted to Dr. W. H. Schultz, Professor of Pharmacology, for valuable suggestions.

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THE PHYSIOLOGIC EFFECTS OF THE ELECTROIONIC ADMINISTRATION OF ADRENALIN*

By C. S. Smith, Ph.D., M.D., and J. B. Brown, M.S., Ph.D., Columbus, Ohio

THE introduction of drugs into the body by direct absorption through the skin is usually considered to be a very slow and otherwise ineffective mode of administration. In this regard Cushny[1] may be quoted as follows: "Drugs are also applied to the skin in order to elicit their general action. Volatile bodies are certainly absorbed by it, although much more slowly than by the lungs or by the stomach and intestine. *Solutions in water of nonvolatile drugs are not absorbed from the skin,* but solutions of certain remedies in alcohol, oils, fats, ether, and some other substances which are capable of dissolving or mixing with the fatty covering of the skin, are absorbed fairly rapidly if they are rubbed in thoroughly." Quite recently Kahlenberg, in a very interesting communication,[2] has shown that such substances as urea, cane sugar, salts and borax diffuse readily through dead skin, which, however, is entirely impermeable to them in the living state. Incidentally he pointed out one noteworthy exception in boric acid, which passes through living skin very rapidly, appearing in the urine within a few minutes after the drug is applied to the surface of the body. In most cases, however, the living skin offers an insurmountable barrier to the passage of drugs into the body by simple diffusion.

The use of the direct electric current to bring about the absorption of ionic substances has been known for some time, but, with a few exceptions, the experimental work on this subject has been very indefinite. In 1921 Inchley[3] showed that atropine, strychnine and aconitine of the alkaloids and calcium and ferrous iron of the metallic ions were forced into and through the skin by the application of a few milliamperes of direct current over varying periods of time. Of the negative ions he proved further that cyanide, salicylate and ferricyanide anions were absorbed, but that the penetration was no greater than one would effect by hypodermic injection. Inchley tried adrenalin with no effect on the blood pressure, which is not surprising, since his solution of the drug did not affect the blood pressure of the rabbit which he was using in the experiment when three minims were injected hypodermically. In a very recent article Pack, Underhill, Epstein and Kugelmass,[4] in some excellently controlled experimental work, demonstrated accelerated absorption by electrolysis with the following substances: Hg^{++}, Mg^{++}, Zn^{++}, Pb^{++}, strychnine, pilocarpine, curare, picrotoxin, veratrine, nicotine, cocaine, procaine, butyn, and a number of the halogens, nonmetals and acids. Benzyl carbinol, salicaine, nitrite, salicylate and caffein were not introduced by this method. It seems certain, therefore, that ionic medication with either a positive or negative ion is a possibility, provided practical uses for these ions and this mode of administration are found.

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We have been interested for some time in discovering some simple means for administering adrenalin, particularly for the relief of bronchial asthma. The only satisfactory method is that of hypodermic or intramuscular injection. While the relief with many individuals thus treated is very rapid and complete, repeated application is inconvenient and in many cases impracticable. There is also some danger of overdosage. It should be further noted that very small doses of this drug are effective in relieving asthma, one to three drops of a 1-1000 solution (0.06 to 0.2 mg.) being usually sufficient. It occurred to us, therefore, that, if adrenalin were absorbed at all by ionic medication, this means of introduction into the body would be very desirable, particularly in the treatment of this disease in chronic cases where frequent use is necessary.

Experimental Part

Introduction

In a preliminary paper[5] an apparatus was described for the electrolysis of an adrenalin solution with deposition of the positive adrenalin ion in the skin. This apparatus was so constructed that, by holding it in the hand and applying the other end of the apparatus to some spot on the body, the circuit was automatically closed. It was simply constructed and could be used conveniently by a chronic asthmatic as often as desired. The area of the positive electrode was about twenty square centimeters. The current was supplied from a small sized radio B-battery, 22½ volts.

In the tests here described a similar apparatus was employed, the only difference being in the size of the positive electrode, which in this case had an area about twice that mentioned above. This was covered with from three to five thicknesses of gauze, which in turn was well moistened with 0.3 per cent adrenalin solution in either hydrochloric or sulphurous acids, sufficient acid being present to keep the solution at a P_H of from 2.0 to 3.0. This low P_H is necessary to inhibit oxidation and coloration of the otherwise unstable adrenalin.

The chemistry of the ionization and electrolysis is as follows: Giving adrenalin chloride the formula, RNH_3Cl, we have the following equilibrium,

$$RNH_3Cl \leftrightarrows RNH^+_3 + Cl^-.$$

In the process of electrolysis the RNH^+_3 gains one electron and is then converted into free adrenalin base and an atom of hydrogen. The depth of penetration of the base is not known. From its effects described below, however, it obviously penetrates as far as the small blood vessels, which it constricts. Whether it diffuses chiefly into the blood vessels and arrives at the circulation by that route, or whether it enters into the lymphatics is not known. Lyon[6] believes that absorption is mainly through the latter channel (with hypodermic injection).

Local Effects

The local effect of adrenalin introduced into the skin by ionic medication is an almost immediate blanching of the area to which the electrode is applied, caused by complete vasoconstriction. The blanching appears in from one to three minutes and persists from fifteen minutes to four hours, depending on

the length of application. The blanched area marks the exact outline of the electrode and does not spread further. In fact, in some cases, in the immediate periphery of the blanched spot, there is a narrow area of hyperemia, suggesting either slight irritation or, more probably, the minimal effect of adrenalin, vasodilatation.

SYSTEMIC EFFECTS

These were determined by taking the systolic blood pressure in the usual manner with a Baumanometer. The subject was caused to lie down during the course of the experiment. A preliminary rest period of at least ten minutes

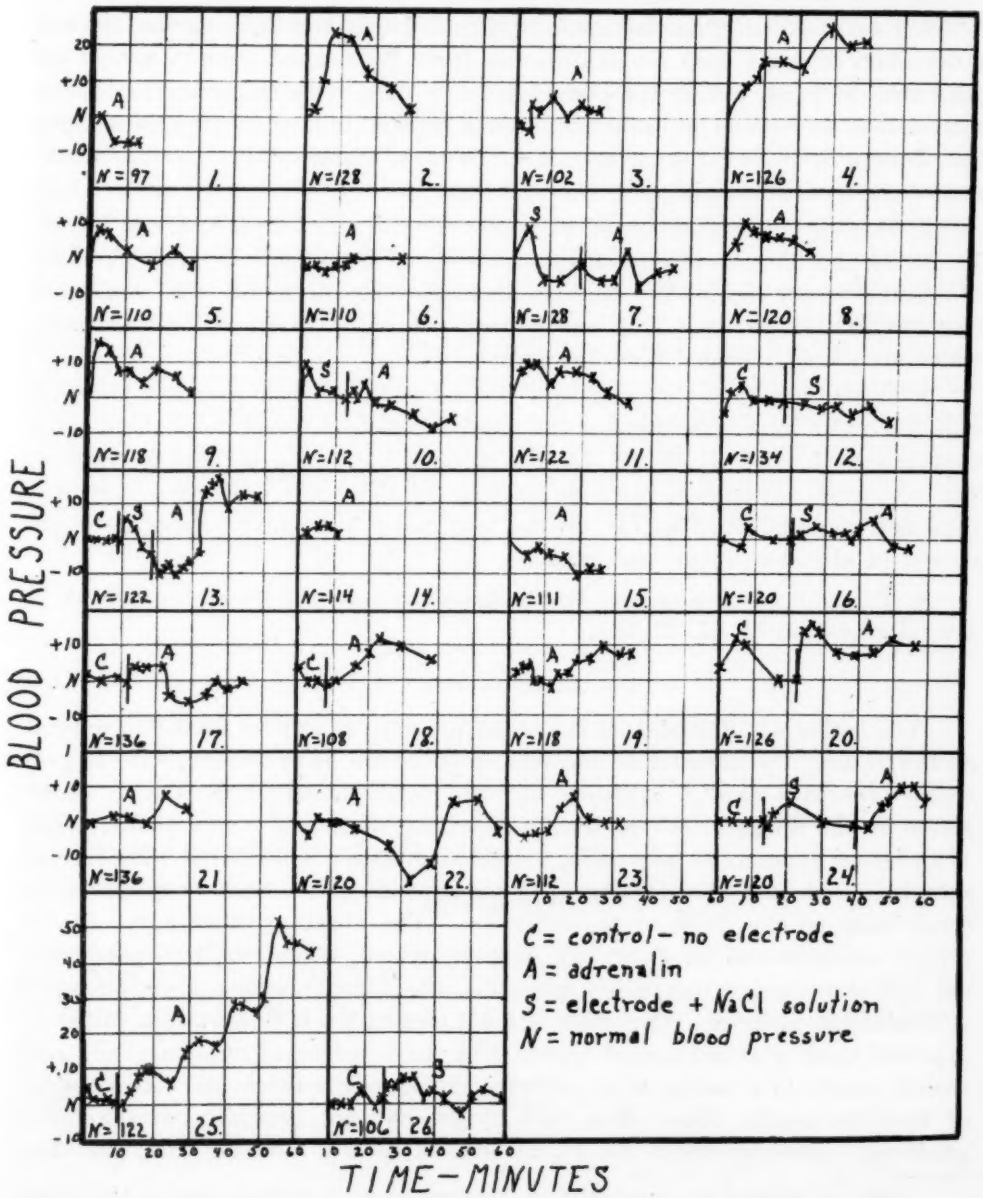

Fig. 1.

was allowed, and then readings were taken over two or three minute intervals until two or more consecutive readings were alike. Then, with as little disturbance as possible, the electrode was applied to the arm, shoulder or chest. Usually several spots of application were made. Controls under like conditions, but with the use of physiologic salt solution at a P_H of 2.0 to 3.0, showed very little if any change from the normal, rarely over five millimeters. The patient was kept as quiet as possible to eliminate the possibility of changes due to excitement. The subjects were, with one exception, men* about twenty years of age. The records of the blood pressure are shown in Fig. 1.

The results of these experiments may be briefly pointed out as follows:

1. Controls(S) in Experiments 7, 10, 12, 13, 16, 24 and 26 in no case showed a deviation of more than ten millimeters from the normal, and in nearly all cases this deviation was on the plus side, i.e., a rise. This indicates that under the conditions of the experiment the electric current alone may produce a slight rise, from 0 to 10 millimeters, and in a few cases a slight fall. In one experiment both conditions existed.

2. During the application of adrenalin, Experiments 2, 4, 8, 9, 11, 13, 18, 19, 20, 24 and 25 gave blood pressure rises of 10 millimeters or greater. This constitutes 46 per cent of the experiments employing adrenalin. The maximum rise was 52 millimeters. This was accompanied by tremors and nervousness, which symptoms persisted for some hours.

3. A number of the experiments showed definite blood pressure lowering with adrenalin, either throughout the experiment, or at some point during the experiment (1, 3, 6, 7, 10, 13, 15, 17, 22, and 23). The maximum lowering was seventeen millimeters, with an average of about five. The lowering was thus not so pronounced as the raising, mentioned previously, although it occurred in almost as many instances.

4. Pronounced raising and lowering occurred in the same experiment in several cases, (3, 13, 17, 22, 23).

DISCUSSION OF RESULTS

The action of adrenalin on the blood pressure is usually stated to depend on the dosage. With minimal amounts of adrenalin the blood pressure is generally lowered. This is explained by Dale and Richards[7] as due to direct action on the lining of the capillaries, resulting in loss of tone and dilatation. With larger amounts of adrenalin, the blood pressure is markedly raised to an extent dependent proportionally upon the amount given, because of profound constriction of the walls of the arterioles. The blood pressure curve of adrenalin administered intravenously shows a sudden, sharp rise, but more gradual fall to normal within a few minutes. The blood pressure lowering curve is similar but reversed. The effect of ionic medication with adrenalin might be expected to show both types of curves, first the lowering of minimal doses, and then a change to a raising as the amount introduced is increased. As a matter of fact the results above show both types, but not generally in the order predicted. Experiments 3, 13, 17, 22, and 23 show a lowering first, and then

*We are indebted to a number of the members of the Sophomore class of the College of Medicine for serving in these experiments.

a rise to a point above normal. The differences between the maxima and minima in these curves varied from 11 to 28 millimeters, which are well out of range of experimental error.* In a number of cases, however, the rise came first, followed by a fall to a point below normal. Furthermore, in some cases only the rise or the fall appeared. From these facts it is difficult, and perhaps not desirable, to attempt to explain these varying actions on pharmacodynamic grounds without additional experimental evidence.

The differences are undoubtedly due to variations in individual reaction towards adrenalin and in the rate of absorption of the drug. This latter would in turn vary directly with any of the conditions affecting the electrical conductivity of the skin or even of the body in general through which the current must pass.

These experiments show in two ways that appreciable doses of adrenalin may be introduced into the body through the skin by means of the electric current. In the first place, in every experiment the local blanching of the skin occurs. The electrode alone with salt does not produce this effect, nor is there any sign of blanching when adrenalin, dissolved in water or in an unctuous medium, is applied to the skin. Likewise with reversal of the polarity of the electrodes the results are negative. All of these facts prove that the electric current is essential in overcoming the natural barrier which living skin opposes to the diffusion of adrenalin chloride. In the second place, in the majority of cases, enough adrenalin finds its way into the system to produce appreciable changes in the blood pressure, either lowering or raising it. While in a few instances this might be ascribed to the effect of the current alone or to individual excitability, in many cases the effect is much greater than we have been able to produce by the current alone. In these cases the effect is due to a general systemic action of the adrenalin.

SUMMARY

The effects of the electronic administration of adrenalin on the human body may be stated as follows:

1. Blanching of the skin occurs at the point of application of the electrode.

2. The blood pressure effects are variable. In almost half of the cases a rise of ten millimeters or more is observed. A rise of as high as fifty millimeters may result. In a few cases there is a distinct blood pressure lowering, thus showing the effect of minimal doses.

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*The limit of error in our readings was not more than ± 1 to 2 mm.

AN APPARATUS FOR THE ELECTROIONIC ADMINISTRATION OF ADRENALIN*

By J. B. Brown, M.S., Ph.D., and C. P. Effler, Columbus, Ohio

An article by Pack, Underhill, Epstein and Kugelmass† on electroionic medication suggested to us the possibility of applying this principle to the administration of adrenalin. Such a mode of administration would overcome the disadvantages and inconveniences of hypodermic injection and would be especially desirable where frequent application is necessary for either local or systemic effects.

With these points in mind we have devised an instrument, a cross section of which is shown in Fig. 1. The metal parts are of nickeled brass. Electrode *A* (−) is of the size and shape of an ordinary door knob. The second electrode, *C* (+), consists of a flat disc, grooved on its outer edge so as to afford a concave

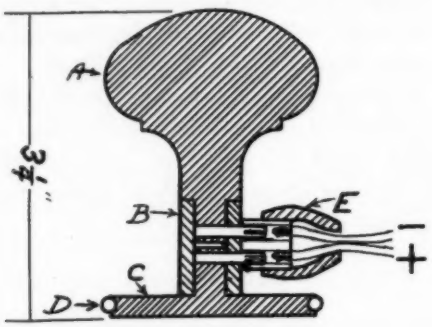

Fig. 1.

pocket for a broken ring of spring brass, *D*, which holds in place several thicknesses of gauze over the lower surface of *C*. The exterior of *D* is lacquered with an insulating material. *B* is a rod of fiber, bakelite or hard rubber which separates and insulates the electrodes. *E* is a small electric plug leading by a cord to a small radio *B*-battery (22.5 volts), and is provided with a third pin, not shown, between *B* and *E*, making it impossible to interchange the polarity of the electrodes after the proper battery connections have been made.

The instrument is used as follows: 4 to 6 circular pieces of gauze of a diameter about one-half inch larger than the disc *C* are placed on *C*, and the spring *D* is pushed on to hold them in place. The apparatus, ready for use, is pictured in Fig. 2. The gauze is well moistened with adrenalin solution (either the chloride or sulphite). The electrode *A* is then firmly held by the patient in one hand and applied to the part desired. In the treatment of asthma

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and for blood pressure effects we have found the arm and shoulder to be satisfactory points of application. In this way the patient completes the electric circuit through his own body, and the positive adrenalin ion is deposited on and absorbed by the negative surface to which *C* is applied. The local effect is chiefly a complete blanching of the skin. The discomfort is practically negligible and consists of a slight tingling in the hand holding the apparatus.

Preliminary experiments have shown that solutions of adrenalin from 0.1 to 1.0 per cent in strength produce the local constriction. The optimum concentration is about 0.3 per cent. The P_H of the solution should be low, preferably from 2.0 to 3.0, in order to increase the stability of the adrenalin and inhibit its discoloration. The instrument is used until the desired effect is

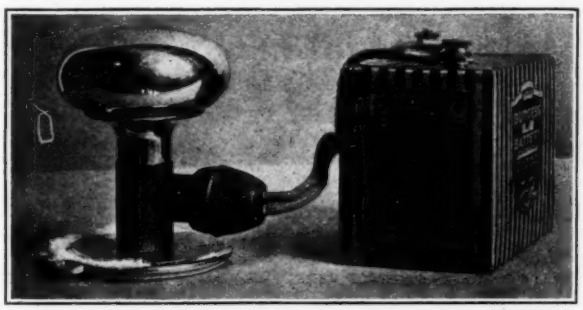

Fig. 2.

obtained, usually from five minutes to an hour. The gauze need not be changed for several days, when the color becomes unsightly. When not in use, the gauze may become completely dry without impairing the adrenalin, in which case it is moistened again with water. One charge, seven cubic centimeters of 0.3 per cent adrenalin, contains about twenty normal intramuscular doses. The charge is shown to be exhausted when the local effect is no longer produced.

A series of experiments upon the blood pressure effects of this method of administration of adrenalin in normal individuals is now in progress. In addition, this method is being tried clinically in selected cases of bronchial asthma. The results of these observations will be reported in the near future.

LABORATORY METHODS

A METHOD FOR THE STUDY OF THE VARIATIONS IN VOLUME OF ORGANS IN THE INTACT ANIMAL*

By Estes H. Hargis, M.D., Rochester, Minnesota

NO comprehensive study of the variations in volume of organs in the unanesthetized animal, other than the limbs, has been made. Many plethysmographic studies are recorded for which the animal was anesthetized, but under such circumstances repeated observations for any length of time are not possible, and no results uncomplicated by the effects of the anesthetic can be obtained.

In a problem on the variation in volume of the spleen in relation to digestion and other processes, in which it was desired to make repeated observations extending over long periods, a procedure which would not be complicated by an anesthetic was necessary; therefore, the method here described was formulated. While the studies on the spleen were of paramount interest, the procedure was also applied to the kidney and gall bladder as a control. A plethysmograph for this purpose must be light, and flexible enough to permit of its being sutured into position and rigid after suturing. It must be of material that can be sterlized, and it must be nonirritating so that it will heal in position. Such an instrument was made of nonflexible collodion. Plethysmographs for the various organs demand special attention only in the construction of the model.

CONSTRUCTION OF THE SPLENIC PLETHYSMOGRAPH

Anatomically the spleen of the dog lends itself to a study of this kind. It is mobile; its vessels and omentum permit it to be drawn to the surface through an abdominal incision so that subsequent procedures are comparatively simple. Its long axis runs obliquely downward and forward, and the greater end or head is nearer the median line and slopes toward the tail, which, however, is somewhat larger than the median portion of the organ. The width is greater than the thickness, and the vessels are given off in longitudinal group along the dorsal surface.

The first step in the construction of a plethysmograph, which can be placed around an organ in the abdominal cavity and heal in position, is to mould from warm paraffin a model of an organ similar to the one to be studied (Fig. 1). Such a model can be made from the spleen of an animal of approximately the same size. As the size of the spleen varies consider-

*Work done in the Division of Experimental Surgery and Pathology, The Mayo Foundation. Received for publication, August 4, 1925.

ably in dogs of the same weight, it is necessary to have two or three plethysmographs of different sizes before attempting to apply one. The model should be approximately one-fourth larger than the organ, which will thus fill about 80 per cent of the completed instrument. Allowance should be made for the contraction of the collodion during the process of hardening. Paraffin is placed in warm water until it becomes soft; then the model is moulded into the desired shape with the fingers (Fig. 2). It conforms to the shape of the spleen in a general way, and is shaped something like a flattened banana. The concave surface represents the hilar or dorsal surface

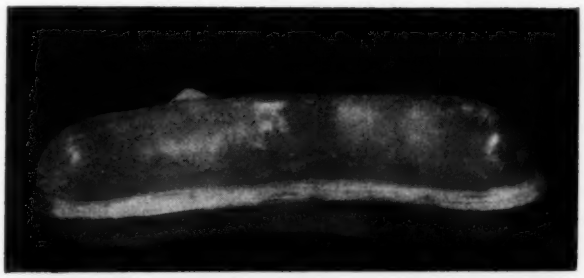

Fig. 1.—Paraffin model to be used in the preparation of a collodion plethysmograph for the spleen of a dog.

Fig. 2.—Splenic plethysmographs of various sizes. One has been opened and sutured, and another is opened along the convex surface to show the interior of the instrument.

of the spleen; the convex, the ventral. The two other faces, flattened longitudinally, will be referred to as the superior and inferior surfaces. Two ends represent the head and tail. When the model of the organ is thus complete, a shelf or ledge, triangular in cross section and with an altitude of 1 cm., equidistant from the superior and inferior surfaces, beginning about 2 cm. from the ends on the convex surface, and running around both ends and the entire length of the concave surface, is moulded from a separate piece of paraffin. This shelf can be trimmed to the desired shape with a knife. A piece of glass tubing, of not less than 6 mm. inside diameter, with relatively thin walls, is introduced into the paraffin deep enough to make it rigid and

allow at least 4 cm. to protrude from the surface. This tube should be placed obliquely to the surface about 5 cm. from the lesser end.

After the model is completed it is placed in cold water and allowed to harden, is then removed and thoroughly dried. Several thick layers of non-flexible collodion are applied to the surface, either by pouring from a suitable container and distributing with the fingers, or by dipping. This should be allowed to remain unmolested until the collodion has become dry and hard. Then three layers of gauze are fitted snugly about the model and successive layers of collodion added to a thickness of approximately 2 mm. The glass tube need not be covered with gauze but added layers of collodion are necessary to make its mould sufficiently rigid after the glass has been broken out.

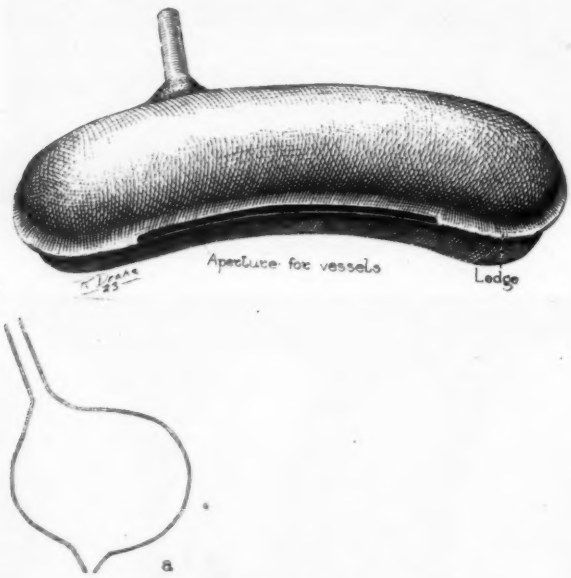

Fig. 3.—Completed plethysmograph showing ledge and aperture for vessels; *a*, cross section of instrument at point of exit of tube.

At least forty-eight hours should be allowed for the collodion to harden. After it has become thoroughly dry and hard an incision about 2.5 cm. in length is made longitudinally along the convex surface, and the model is placed in a bath of boiling water until the paraffin has melted out. The plethysmograph is taken from the water-bath while hot and rubbed vigorously with gauze to remove any paraffin adhering to its surface, and, if necessary, is immersed in a second bath of boiling water. After it has cooled for a time, the glass tube is broken out by placing it on a metallic surface and tapping gently with any small instrument. The plethysmograph should be opened with a sharp knife along the apex the entire length of the ledge, and the edges on either side of the opening cut away with scissors, beginning about 2 cm. from either end on the concave side, to provide an aperture of sufficient width to accommodate the vessels (Fig. 3). A distance of 2 or 3 mm. on each side of the slit is sufficient for this. The sharp edges are covered with collodion and allowed to dry. A thick-walled rubber tube, about 10

cm. in length, is fitted to the collodion tube of the instrument, and if there is any doubt about its remaining in place it may be sutured with a small needle and silk. The small opening on the convex side of the instrument should be sutured and covered with a layer of gauze and several layers of collodion. The plethysmograph is now ready for sterilization and may be boiled repeatedly without injury.

METHOD OF APPLYING PLETHYSMOGRAPH TO THE SPLEEN

All operative procedures are performed under complete anesthesia and rigid sterile technic. Plethysmographs of at least three sizes are sterilized by boiling, and two sterile test tubes are filled with collodion to be used during the closure of the instrument. The abdomen is opened through a left rectus incision and skin towels are applied. Although obvious, it may not be amiss to emphasize the need for the utmost care in introducing into the abdominal cavity a foreign body which is to be left for a long time if it is not to cause symptoms or discomfort. If moderate care is exercised good results will be obtained. The spleen is exposed, drawn out of the wound, and surrounded with warm saline packs, and a layer of omentum is fitted around it. A plethysmograph of appropriate size is selected and the omentum-covered spleen is introduced. The splenic omentum and vessels are arranged so that they are well inside of the space provided for them. The spleen, in the contracted state which exists during operation, should fill about eighty per cent of the instrument and the cut edges of the plethysmograph should fit snugly around the splenic omentum, without, however, any encroachment on the lumen of the vessels. If the size of the plethysmograph is satisfactory and the vessels are properly cared for, the instrument may be closed; this is most easily accomplished by the use of silk and a small needle with a cutting edge.

The method of suturing is optional, but whatever method is chosen, the first suture at each end should be placed at the edge of the splenic omentum in a manner to produce a snugly-fitting angle. The sutures are carried toward and around the ends as far as the instrument has been opened. It is rarely necessary to place sutures through the portion receiving the omentum and vessels unless there is an ill-fitting area, in which case one or more interrupted sutures may be used to approximate the edges, care being taken not to injure the vessels. When the suturing has been completed the instrument is thoroughly packed off with large gauze sponges, and several layers of collodion are applied to the suture line and allowed to dry for several minutes. A thin strip of gauze is placed over this surface and in turn covered with collodion and allowed to dry.

The omentum is drawn around the plethysmograph in such a manner as to enclose it completely, leaving no gaping edges or openings, and is gently sutured in place with 00 catgut. The instrument is placed in the abdomnal cavity and the rubber tube brought to the surface through a stab wound high on the left side; in this position it is more difficult for the animal to remove it, but at the same time the position must not cause torsion of the vessels and interfere with the circulation of the spleen. Tension is exerted

on the tube from the outside to draw the plethysmograph firmly against the abdominal wall so that adhesions may form. Unless this precaution is observed infection may later creep in around the tube. At least one layer of nonabsorbable suture material is used in closing the wound. The rubber tube is cut about 5 cm. from the margin of the skin and left open.

Precaution must be taken to prevent the animal from removing the tube. A wide, closely-fitting metal collar has been found quite satisfactory, and causes the animal no discomfort. A week should be allowed before plethysmographic observations are made. By this time, if care has been taken, there is no danger of leakage from the plethysmograph into the abdomen.

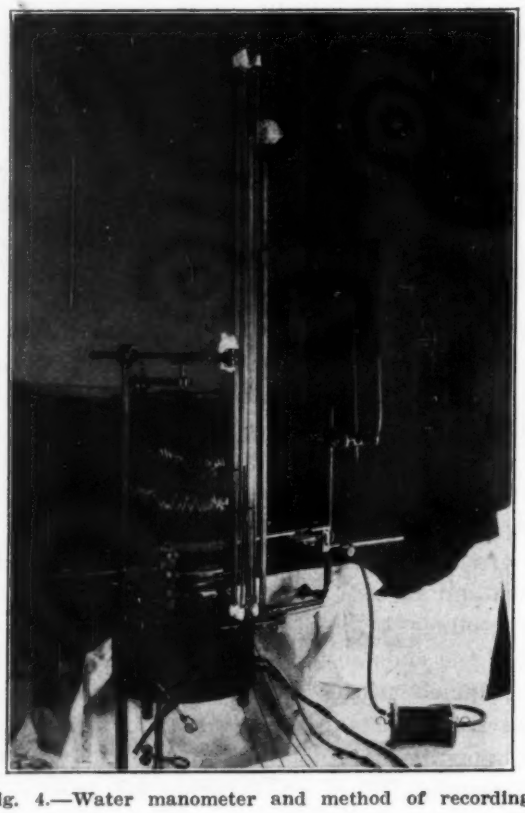

Fig. 4.—Water manometer and method of recording.

METHOD OF RECORDING OBSERVATIONS

Observations are made only on animals that have been thoroughly trained for the purpose. They receive no anesthetic or drugs, and suffer no discomfort. At the end of the first postoperative week they are placed under preliminary observation for increasing periods of time so they may become accustomed to the surroundings and acquire the habit of lying quietly on the table. They are easily trained, and learn the routine quite readily; the trained animal will remain perfectly contented for long periods, frequently sleeping while the observations are being made. The animal is placed on his side upon a concave holder lined with soft mats, a collar attached around

the neck, and the feet restrained lightly. Appropriate connections are made between the tube from the plethysmograph and the recording apparatus.

The water manometer, with a special method of recording, has been chosen as the best method for recording changes in volume of the spleen. The tambour has been used with very good results, but its use has some disadvantages which are not encountered in the former method. It is essential to have a method in which there is as little resistance as possible in the recording apparatus, so that it will react quickly to changes and will be capable of recording the delicate variations in the volume of the organ. A method of recording to be used with the water manometer[1] (Fig. 4) consists essentially of sections of glass tubing, with an inside diameter of 3 mm., placed in a brass frame so that each section is in front of a vertical column. On each of the three similar columns there is a delicate carriage which may be moved up and down, having on the drum side a writing point and on the

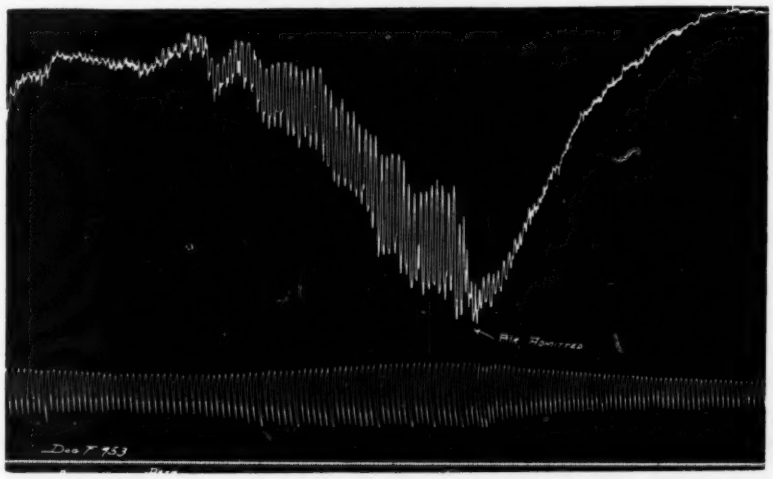

Fig. 5.—The effect of rebreathing on the volume of the spleen in the intact animal is obtained by the method described. The upper curve is splenic volume; the median curve, respiration recorded by the respiratory tambour, and the lower curve, the time in seconds.

observer's side a pointer which is to be kept on a level with the meniscus of the column of the fluid by manipulation of the carriage with two cords. The recording instrument is held in position by clamps and so placed that the writing point rests on the revolving surface of the smoked drum. This method of recording has proved very satisfactory and one is able to obtain consistently records which are clear and accurate (Fig. 5).

DISCUSSION OF METHODS

By the methods formerly used studies on the changes in volume of the internal organs were difficult or impossible because results could not be obtained without being complicated by the effects of an anesthetic; the results of external stimuli could not be investigated, because each experiment was an acute experiment and the animal could not be studied for any extended

[1]Potter, J. C.: A Mechanical Method for Recording Small Variations in Pressure and Volume, JOUR. LAB. AND CLIN. MED., 1924, ix, 648-650.

period. The application of the collodion plethysmograph has overcome many of the difficulties and given it a wide range of usefulness. The changes that occur in the normal physiologic processes of the intact animal under the influence of different diets, drugs, and stimuli may be studied for long periods in the same animal when no anesthetic is used.

The method requires a well regulated operating room and a rigid technic. Then, too, it requires a certain amount of skill to make and apply the plethysmographs and obtain the best results. Even though the organ is covered with omentum, infection may at times creep in and cause thickening of the capsule, and thereby decrease the range of the variations. Moreover, precautions must be taken to prevent the animal from removing the tube for a time following operation, and observations can only be made on selected animals which have been trained for the work.

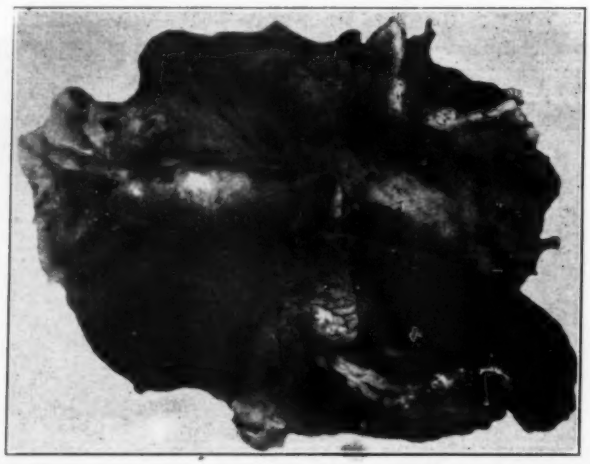

Fig. 6.—The spleen of a dog which was placed in a plethysmograph and studied for two weeks and removed to observe the changes in the organ and encapsulation obtained. At the lower median portion of the organ is shown an area of thickening which occurred as a result of a small tear in the omentum covering it. The encapsulation is very thorough, as may be observed from the cut edges of the thickened omentum which was placed around the outside of the instrument shown in the upper median portion.

The type of plethysmograph described has been used in a study[2] of the effect produced by diets, certain drugs, rebreathing, and various stimuli on the volume of the spleen in the intact animal. The spleen was studied while intact and also after the nerves had been severed. After the organ had been divided each half was placed in a separate plethysmograph, the nerves to one-half being severed, and the others left intact. Tracings of the variations in volume of the two portions under various conditions were obtained concomitantly.

Plethysmographs have been made for the kidney and gall bladder and have been applied with satisfactory results, although no extended study has been made on either of them, other than the mere application of the method so as to provide controls for the study of the spleen.

[2]Hargis, E. H., and Mann, F. A.: A Plethysmographic Study of the Changes in the Volume of the Spleen in the Intact Animal, Am. Jour. Physiol., 1925, lxxv, 180-200.

A COMPARATIVE STUDY OF LIVER FUNCTIONAL TESTS*

By A. I. Rubenstone, M.D., and Louis Tuft, M.D., Philadelphia, Pa.

RECENT medical advances have created a demand for functional tests, so designed that when applied to the organ tested they will serve as an index of the amount of function present and thus detect deviations from the normal. The application of such a test is fairly easy in organs which have a readily accessible secretion or excretion and which carry out but a single major function as, for example, the stomach or kidney. With the liver the problem is more complex, chiefly for three reasons:

1. The necessity for an indirect method of studying its function, either through the blood or bile.

2. There exists in the liver, as in most organs, a large margin of safety; thus Rous[1] has shown experimentally that only one-fourth of the liver is necessary to maintain normal function.

3. Finally, the liver is possessed of multiple functions about which comparatively little is known. The known functions concern themselves chiefly with metabolism of carbohydrate, protein, fat and iron, the secretion of bile, and in filtering from the system noxious irritants, particularly foreign proteins.

In an organ possessed of so many varied functions it is naturally difficult to have a single test serve as an index of its total function. In the past many tests have been suggested, each dependent on some abnormality in one of the hepatic functions. Many of these have been discarded, because they were either unreliable or too cumbersome for clinical use. With recent advances in blood chemistry and more frequent use of the duodenal tube, others have been so modified as to make them clinically adaptable.

For more than two years in the laboratories and medical wards of the Mt. Sinai Hospital of Philadelphia we have been investigating hepatic function tests, selecting those which were being used in other clinics with apparent success. Each test was first tried out individually; later comparative studies were made in order to ascertain their relative value. It is the purpose of this report to record briefly the results of our experiences with these tests based on studies performed on more than 150 individuals, normal and abnormal. For the sake of brevity statistical data will be omitted in this report, and technical details will likewise be curtailed. Five different types of tests were studied:

1. Hemoclastic Crises of Widal.
2. Levulose Tolerance Test.
3. Icterus Index and van den Bergh Test.
4. Dye Tests.
5. Nitrogen Partition Studies.

*Read before the Fourth Annual Convention of the American Society of Clinical Pathologists, May 20 to 23, 1925.

HEMOCLASTIC CRISES OF WIDAL

This test is intended to investigate the albumose-storing or proteopexic function of the liver. Widal[2] found that in liver dysfunction instead of the usual leucocytosis after ingesting 200 c.c. of milk, a leucopenia occurred, beginning in about twenty minutes and often lasting from an hour to an hour and a half. He attributes this reaction as due to an overflooding of the circulation by the albumose, which the liver was unable to store up and which, when liberated in the blood, produces anaphylactic shock with fall in blood pressure and leucocytes.

Our experience with this test has not been very satisfactory. Results were variable and often difficult of interpretation; thus blood pressure estimations were found so unreliable as a result probably of nervous or psychic influences that we have latterly discarded this part of the test and used only the leucopenia to indicate a positive reaction. Though in patients with gross liver damage, as in carcinoma, positive tests were obtained, in other patients with evident liver dysfunction as, for example, acute catarrhal jaundice, negative reactions occurred. Furthermore, positive tests were obtained in conditions with no evidence of liver dysfunction, such as arthritis or eczema.

From these observations we agree with Feinblatt[3] and others that this test is useless in quantitatively measuring liver damage. Its relation to conditions of allergy and vagotonia, as suggested by several authors, warrants further investigation.

LEVULOSE TOLERANCE TEST

This test, arising as a modification of the original levulosuria test of Strouse,[4] is dependent on the fact that levulose, in contrast to glucose when ingested, produces very little rise in blood sugar, the rise being seldom of more than an hour's duration with return to normal or usually below within two hours. This is attributed to the fact that it is normally more rapidly converted into glycogen and stored in the liver than is glucose, hence little of it finds its way into the general circulation. When the liver storing power is interfered with, as in disease, some of the levulose will filter through and produce not only a blood-sugar rise but also a delayed fall.

The favorable reports of Spence and Brett[5] in toxic hepatitis following salvarsan and that of Covell[6] in patients with amebic abscess, encouraged us to determine the merits of this test. It is performed in the same manner as a glucose tolerance test except that less levulose is ingested, from 35 to 50 gm., depending on the patient's weight.

The results of our studies with this test are interesting. In our normal controls the average blood-sugar rise in an hour was 10 mg. and in two hours it was at, or usually below, normal. With the exception of diabetes, when a similar curve is obtained as with glucose, conditions with gross liver dysfunction such as carcinoma, toxic hepatitis, advanced cirrhosis, or marked cardiac decompensation, produced an average blood-sugar rise of 30 mg., and in all there was a delayed return to normal lasting three hours or longer. In patients with mild dysfunction as in cholecystitis, catarrhal jaundice, etc., the

curve was either normal or showed only a mild rise, although in most of these there was a delay in the return to normal until the third hour.

These observations lead us to believe that this test may serve as an index of the carbohydrate function of the liver. So little is known of the mechanism of this function that we are handicapped in correctly interpreting its usefulness. It is likely that in conditions in which there is but slight or moderate disturbance in the carbohydrate function, enough readjustment may take place through the agency of other factors, either hepatic or extrahepatic, that, as a result, there may be little or no change in the levulose curve, the latter being at present the best means of detecting such disturbance. On the other hand, in advanced disease such as carcinoma, where the liver is so damaged that readjustment in the carbohydrate function cannot keep pace with the extent of impairment, the levulose curve will show changes commensurate with the degree of damage. Obviously this test can be of clinical assistance only in marked liver damage but is of little or no aid in milder hepatic dysfunction, where a functional test is most desired. We cannot recommend it for general use as a practical liver function test, but we believe that it is the best means we have at present for studying the carbohydrate function of the liver.

ICTERUS INDEX AND VAN DEN BERGH TESTS

The normal color of blood serum is due to the presence of serum bilirubin which normally is kept fairly constant in amount by a regular rate of red cell destruction. In various diseases of the liver the bilirubin content of the blood is markedly increased, resulting often in frank icterus. Between the normal and the point at which frank icterus occurs is a zone often called the zone of latent icterus or subicteric zone, in which the bilirubin concentration, though increased above normal, is not sufficient to cause definite jaundice. This increase in bilirubin may be due either to conditions causing increased hemolysis, as in pernicious anemia, or to disturbances in the liver interfering with biliary output, as in cholangeitis and others.

The quantitative estimation of serum bilirubin is best performed by the method of either van den Bergh[7] or Meulengracht,[8] the latter with slight modifications being accurate and easy to perform. It consists of matching the serum in a colorimeter against a known standard of 1-10,000 potassium dichromate. The standard is set at 15 and the serum matched against it. The quotient obtained by dividing the reading of the serum into 15 represents the index of the bilirubin concentration and to this number is applied the term icterus index.

Normally the index varies between 4 and 7. Between 7 and 15 is the zone of latent icterus, and above 16 frank jaundice occurs, higher values being obtained, depending upon the degree of jaundice.

In January, 1924, Bernheim[9] reported results of the use of this test in many varied conditions. She found it of considerable value both from a diagnostic and a therapeutic standpoint; it was high in pernicious anemia but low in secondary anemia; high in duodenal ulcer, low in gastric ulcer, and thus served as an aid in differentiating these conditions.

Our experience with this test agrees with that of Bernheim, particularly in hepatic conditions where it serves to indicate the extent of impairment in biliary function and also the response to treatment. Thus, in jaundice with a high index, decrease in the index denoted improvement before changes in the color of the skin were detectable. Patients with cholecystitis had indices varying from normal up to fifteen or more, the amount of increase above normal indicating the extent of impairment of the biliary function. The index was increased in hepatic cirrhosis, being marked in the biliary types and only moderate in the portal and cardiac types. Malignancy of the liver always produced a high index, the extent being dependent upon the amount of hepatic involvement and the presence or absence of jaundice; thus the highest index in our series was present in a patient with advanced hepatic carcinoma with intense jaundice and an index of 150.

It is evident from the above results that the icterus index may serve both as a useful clinical index of the extent of impairment of biliary function and also as a quantitative measure of the progress of restoration of this function, particularly in jaundice. In the latter condition we have been considerably aided by the van den Bergh test used in a qualitative way to differentiate functional or hemolytic from obstructive jaundice. In this manner, by a combination of these two tests, one quantitative and the other qualitative, we have obtained considerable information concerning the biliary function of the liver.

DYE TESTS

The phenoltetrachlorphthalein test of Rosenthal[10] needs little description or comment, much having already been written concerning it. We have followed the technic originally described by Rosenthal with only slight modifications: 5 mg. of the dye per kilogram body weight were injected and the percentage present in the serum at the end of fifteen minutes, one hour and two hours (if indicated) was determined, using the colorimeter devised by Rosenthal. In cloudy sera or in sera with traces of hemolysis, we have used acetone to extract the color, as suggested by Bloom and Rosenau.[11]

Early in our experience we found that the injection of 40 to 50 c.c. of a solution containing 5 to 8 c.c. of dye, particularly in patients with marked jaundice, cachexia or cardiac disease, caused at times severe reactions, but this has been obviated by the injection of the concentrated dye. Our results with this method have been uniformly good. The percentage of dye retention was proportionate to the degree of liver dysfunction, reaching up to 35 per cent in the severe types. Thus the test seems to be a useful clinical index of hepatic function. It is not, however, without its drawbacks: first, the injection of so much dye into a patient whose liver is already damaged is not always safe. Furthermore, since it never reaches concentrations in the blood stream over 35 per cent even in the severest cases, its greatest value is in the severest grades of hepatic involvement. Finally, as pointed out recently by Maurer and Gatewood,[12] the dye is not wholly eliminated by the liver.

Appreciating these facts, Rosenthal studied other dyes and recently proposed bromsulphalein,[13] which is nontoxic, is more rapidly eliminated by

the liver, and, if retained in the blood as a result of liver impairment, is in much higher concentration, the percentage being used as a numerical index of the extent of liver dysfunction. The technic of the test is so simple and safe as to lend itself readily to clinical use; only 2 to 3 c.c. of the dye need be injected and it is necessary only to draw the blood at the end of thirty minutes to obtain the result. Normally no dye should be present in the blood at thirty minutes; the percentage of dye retained at this time represents the extent of impairment of liver function.

Our experience with this newer method of Rosenthal is too brief to warrant conclusions but even this has been sufficient to prove its great superiority over the tetrachlor method and to indicate that it is a valuable test well adapted to clinical use. Whether or not it will serve to indicate minor variations in hepatic function remains for further investigations to disclose.

We must not forget that any dye test simply measures the ability of the liver to extract this foreign substance from the blood stream and get rid of it in the bile; it is in reality a test of liver permeability and not of its total function. For example, even though the dye test may show 80 per cent impairment, it is possible that the carbohydrate or nitrogenous functions may be only slightly affected; in this instance the 80 per cent may give a false impression. We cannot therefore agree with the statement of Rosenthal that the percentage of dye retention measures the extent of liver impairment; it simply represents the degree of impermeability which may in turn be taken as a rough index of impairment of total function, since it is highly probable that any damage to the liver cells will result in impairment of the permeability.

NITROGEN PARTITION STUDIES

A low percentage of urea nitrogen with comparatively high percentages of ammonia and amino-acid nitrogen in the urine as compared with normal urinary nitrogen partition figures, was considered by earlier investigators as evidence of organic liver damage. Similar attempts were later made to study in a comparative way the urea, ammonia, amino-acid, and nonprotein nitrogen content of the blood. Normally, urea nitrogen makes up from 40 to 60 per cent of the total nonprotein nitrogen of the blood; when it falls below 40 per cent, and particularly if there is coincident increase in amino-acid and ammonia nitrogen, it is indicative of liver damage.

The value of blood nitrogen partition studies in patients with liver disease has hitherto received scant attention, judging from the paucity of literature on this subject. Our experience with this method has been confined chiefly to the estimation of urea nitrogen as compared to the total nonprotein nitrogen in patients with various liver conditions, and no attempt has been made to study amino-acid and ammonia nitrogen figures in these patients. In patients with advanced liver disease, low urea and comparatively high nonprotein nitrogen values were obtained, but, in patients with lesser grades of hepatic disease, the proportion was usually within normal limits. It is evident from this that, as with the levulose tolerance test, studies of blood nitrogen partition will be of little value from a practical standpoint

clinically, and, even where gross organic damage exists, the technical details necessary for its performance hardly warrant its clinical use. It is possible that similar factors of readjustment as discussed under levulose are at work here and only when the damage is too great to be overcome by other factors does it show itself in the partition figures. So little is known of the mechanism of protein metabolism that we believe it of value to continue such studies in patients with various hepatic conditions, and, even though it may be of little value clinically, it may help to solve some of the complex problems of liver physiology.

COMPARATIVE STUDIES

In addition to trying out separately each of the above-mentioned functional tests, we have attempted to compare these tests by carrying out as many of them on the same patient as was possible, being careful to do them closely together in order to preclude any variability in liver function. Our results bear out in a general way the conclusions drawn above concerning each individual test. Probably the greatest similarity in quantitative results existed between the icterus index and the dye tests, particularly in mild dysfunction. This is readily explained when one considers that both of these tests are really dependent on liver permeability. The levulose tolerance and dye tests corresponded fairly well but only in those patients in which marked organic damage to the liver existed. In the milder cases the levulose tolerance often gave a normal curve or showed only a slight delay in return to normal.

We do not believe that this is the fault of the test. It is highly probable that in these patients there exists little damage to the carbohydrate mechanism or possibly its damage has been adjusted by other factors. So little is known as yet of the actual mechanism of carbohydrate metabolism that this must, of necessity, be speculative. The facts given concerning the levulose tolerance apply in a general way to tests for disordered protein metabolism in the liver. Only in gross organic liver disease does one find differences between nonprotein nitrogen and urea nitrogen such as has been described as probably indicative of protein dysfunction. In minor degrees of dysfunction these methods do not detect any deviations. This is also true to a certain extent with the hemoclastic, but here we have an additional factor in that sometimes positive reactions are obtained in patients who show no other evidence either clinical or by other functional methods, of hepatic dysfunction.

CLINICAL FORMULA

As a result of these studies with the various tests, it seemed to us that in order to obtain a complete idea of the hepatic function present in any patient, it is necessary to carry out on that patient all these tests, since each concerned itself with a different function. The objections to such a procedure are at once obvious, the annoyance to the patient, the possible difference in function if the tests are done on different days, etc. It occurred to us therefore that if these tests could be combined in such a manner as to be done at one time

without losing their sensitivity it might yield valuable information and make for a complete study. The following procedure was accordingly adopted:

The patient is prepared as for a levulose tolerance; on the same tray the calculated amount of dye contained in a syringe is also prepared. Blood is then withdrawn into two tubes, one citrated or oxalated, and one a plain tube. Enough blood is withdrawn to suffice for all these tests. Through the same needle the calculated amount of dye is injected. Immediately following this the patient is instructed to drink the levulose solution. Blood is then withdrawn from a vein of the opposite arm at thirty minutes, one hour, two hours and, if necessary, three hours after the injection, the blood being again drawn into plain and oxalated tubes as indicated. The blood is allowed to clot, is centrifuged, and the serum is pipetted off. The serum collected before the injection is then used for the icterus index determination and also as the standard for the dye test. As a rule 2 to 3 c.c. of serum is sufficient for this purpose. The oxalated blood collected before injection is used for the urea nitrogen, nonprotein nitrogen, and sugar determination. Blood sugars are then done on all the bloods (oxalated) taken subsequent to injection, and the sera are used to determine the dye retention as described in the Rosenthal method.

By this procedure we are able to carry out the icterus index, blood chemistry, levulose tolerance and dye tests, at one time and thus gain information concerning the bile-pigment, excreting functions of the liver and also its carbohydrate and protein metabolic functions.

Our series of combined tests, though small, has been sufficient to prove that by this method complete insight into the total known hepatic functions may be obtained and properly evaluated. Although at present from a diagnostic standpoint the dye tests and icterus index are most valuable in estimating to some degree liver dysfunction, yet by studying hepatic conditions by the simultaneous combined method, we hope to collect enough data for later report, tending to arrive at a better understanding of normal and abnormal hepatic physiology.

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DISCUSSION

Dr. Ralp G. Stillman.—I think Dr. Rubenstone emphasized the difficulty of getting any satisfaction in such an active organ as the liver. In all of these functional tests we have to have a very marked reduction in function before any of the tests we have will indicate it. None of the tests that we have made have been wholly normal in carcinoma involvement of that organ.

A SIMPLE COMBINATION DISTILLATION AND AERATION APPARATUS FOR THE MICROESTIMATION OF NITROGEN*

By Robert F. McCrackan, A.M., Emanuel Passamaneck, B.S., and Kate E. Harman, B.A., Richmond, Va.

MANY papers have been published recently containing suggestions for overcoming difficulties inherent in the original Folin and Wu[1] micromethods for determining nitrogen. The troubles most frequently mentioned are: bumping and formation of silica during digestions; bumping, frothing, and drawing back of the distillate during distillations; and cloudiness and variation in shades of colors after nesslerization. Folin,[2] himself, has suggested centrifuging, to get rid of turbidity, preparatory to direct nesslerizations, and he advises that test tubes be thoroughly dried to reduce the danger of bumping during digestions.[3]

Watson and White[4] devised a special delivery tube to prevent the froth from urease solutions passing into the distillates. Youngburg[5] advocates the use of "open delivery tubes" in microdistillations as a means of preventing distillates being drawn back into distillation tubes. Moore and Jones[6] recommend passing air from an inflated rubber bag through the urease solutions during distillation, and through digestion mixtures until half evaporated. Boggs and McEllroy[7] pass a slow current of air through the descending part of the delivery tube during distillations. Weathers and Sweany[8] have described apparatus for running eight aerations at once. Wishart[9] proposes the use of beads in a constricted urease tube as a remedy for frothing, and he admits sodium carbonate into the distillation tube through a special tube after the distillation is well advanced. Horvath[10] uses a safety tube with a pinchcock that can be opened when the distillate is being drawn back. Hindmarsh and Priestley[11] claim they had no trouble from frothing when they caused a current of dry air to impinge on the surface of the borax solution to drive the ammonia over in a urea determination on 0.1 c.c. of blood. Osborn[12] describes apparatus for completing four digestions at once, after having carried each separately to the point where the mixture turns brown. Johnson[13] proposes the use of a "flutter valve" to prevent the drawing back of distillates. Parnas and Heller[14] propose the distillation of the ammoniacal solutions under reduced pressure with steam, and that condensations be made with a silver cooler. Stanford[15] uses a closed vacuum system and brings about distillation at about 35° C. Koch and McMeekin[16] propose modified reagents for digestions and for direct nesslerizations. Feinblatt[17] thinks satisfactory results can be obtained by the nesslerization of urease solutions without distilling.

The apparatus described here has been tried out by about a hundred students, teachers, and clinicians, over a period of about two years. It has been

*Contribution from the Medical College of Virginia, Laboratory of Biochemistry. Received for publication, October 19, 1925.

found satisfactory because of its simplicity, and because it eliminates so many troubles, and makes it possible for people of medium ability to get trustworthy results without a great deal of practice. The apparatus is designed to obtain the ammonia from an ammonium salt by distillation, or aeration, and with a view to estimating it quantitatively in the distillate.

An Erlenmeyer flask, *A*, Fig. 2, clamped in position on wire gauze, and filled a third or less full of distilled water containing one or two drops of concentrated sulfuric acid, is used as a steam generator. It has a safety tube, *B*, at least thirty inches long, which is slightly constricted at its lower end. A test tube is sometimes hung over the upper end to prevent the entrance of dust. A delivery tube, *D*, constricted a little at its lower end, leads through the rubber stoppers, *C* and *E*, from flask, *A*, into a tube or flask attached to rubber stopper, *E*. Similarly a second delivery tube, *F*, with a much constricted outlet, leads through rubber stoppers, *E* and *G*, into dilute acid in a tube or volumetric flask, *I*, which is attached to rubber stopper, *G*. The tube, *H*, that leads from *I*, may

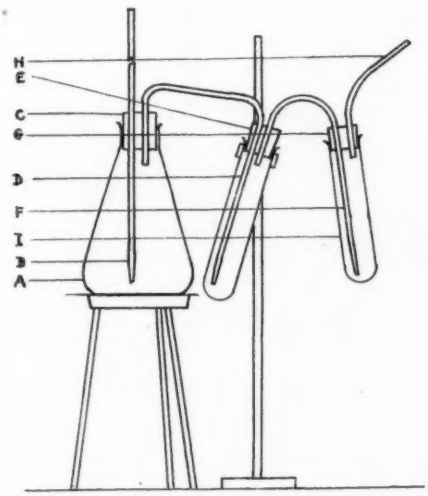

Fig. 1.

be attached to a pump, if the apparatus is to be used for aerating. It can be further improved as aeration apparatus by making the delivery tube, *F*, from one of Folin's thirty cm. aeration tubes, which ends in a bulb with numerous perforations, and it can be still further improved by causing the air entering flask *A*, to pass through another of these tubes substituted for tube *B*. This can be accomplished by removing *B*, or by capping *B* with a piece of rubber tubing plugged with a glass rod and having the Folin tube pass through a third hole in stopper, *C*, into the dilute acid in flask *A*. In such an arrangement it would only be necessary to remove the rubber cap from one of the tubes leading into flask *A*, to the other, to change an aeration outfit into one for distillation, or vice versa.

It is the practice in this laboratory to follow Folin's methods with very little variation, except that the ammonia formed in total nitrogen determinations is distilled before nesslerization. Five c.c. of ten normal sodium hydroxide (40 gm. per 100 c.c.) is used to liberate the ammonia in a total nitrogen deter-

mination, and saturated borax, as directed by Folin, in urea determinations. The usual 75 c.c. test tube has been found satisfactory from which to distil the ammonia from a digested mixture, but a flask is better where the ammonia has to be distilled from a urease solution. This is because of the excessive frothing. When a test tube is used, Fig. 1, the delivery tube, *D*, may be bent in such a way as to cause the bottom of the test tube to rest on the edge of the gauze that supports the flask *A*, making it possible to heat both with one burner. When a flask is used on stopper *E*, results are more liable to be high, because of the greater amount of ammonia in the air. If the flask is large, or if ammonia fumes in the laboratory air are appreciable, it may be found desirable in accurate work to acidify the ammonium salt solution, if it is not already acid, so as to insure retention of the ammonia, and then to displace the air in the flask with steam from generator, *A*, then to block the tube, *F*, with a tube of distilled water, while the flask cools and fills up with ammonia-free air from flask *A*.

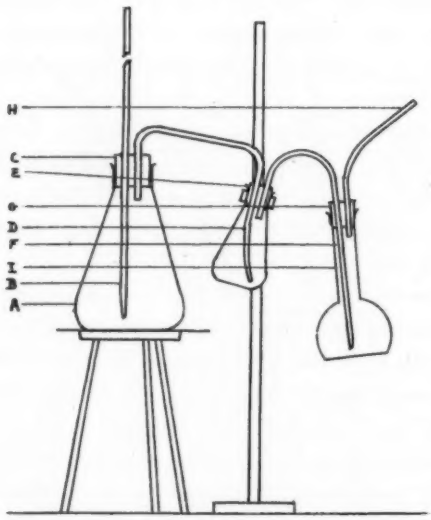

Fig. 2.

The receiver containing an acid solution can be attached at *G*, and the ammonia be liberated and distilled in the usual way.

The advantages to be obtained in the use of this apparatus are:

1. It eliminates bumping and back-suction during distillation.

2. It reduces the frothing evil of the urease method, as a larger container than a 75 c.c. test tube can be used, and the burner may be removed at any time without spoiling a determination.

3. Constrictions in tubes, *B* and *F*, or bulbs at their ends, with perforations, insure better absorption of ammonia from air bubbles.

4. Distillation removes most of the causes of cloudiness in nesslerized solutions. The troublesome silica, dissolved from the container during digestion, the salts resulting from the nesslerization of concentrated acids, the catalyzer, and magnesium compounds that Folin thinks largely responsible for clouding during nesslerization,[18] are left behind, so that the distillate unknown can be

nesslerized exactly the same way as the standard known. It is unnecessary to add anything to the known before nesslerizing to insure the same shades of color and parallel conditions in the known and unknown, and only a third as much Nessler's solution is used as when digestion mixtures are nesslerized directly. Indeed, Folin's own ideal conditions for using 10 c.c. of Nessler's solution per hundred c.c. of nesslerized mixture, in the absence of interfering acids and alkalies, are obtained.

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ACETONE METHYLENE BLUE—METHYLENE AZUR EOSIN SOLUTION FOR STAINING BLOOD SMEARS*

By Frederic Proescher, M.D., and Albert S. Arkush, A.B., Agnew, Calif.

METHYL alcoholic solutions of eosinic acid methylene blue (Jenner, May-Grünwald) in combination with methylene azur (Leishman, Wright) are the commonly employed routine blood stains. The commercially prepared stains may vary greatly in their staining properties because of the difficulty in preparing an eosinic acid methylene blue methylene azur of constant proportions. Different batches vary greatly in their staining effects. McNeal has lately advocated a methyl alcoholic solution of eosin, methylene azur, and methylene violet (tetra chrome) as a substitute for Leishman's stain. While this stain is fairly good, it cannot compare with a good Leishman or Wright stain.

We have tried various combinations of eosin, methylene blue, and methylene azur in methyl alcohol and acetone. By using acetone (and water) as a solvent, we find that the granules are excellently stained and precipitation of the dye is reduced to a minimum.

The stain† is prepared by mixing equal parts (about 60 mgms. each) of methylene blue (Merk), thiazin red (methylene azur**), and eosin with 25 c.c. water, and then adding 50 c.c. acetone. The acetone should be C.P., Mallinckrodt's being used by the authors. Toluidin blue may be used as a substitute for methylene blue. Gruebler's eosin (extra BA) should be used if obtainable. The domestic eosin Y. of the National Aniline and Chemical Company is not so good, though usable. By substituting orange G. for part (about ⅓) of the eosin, the eosinophilic granules are made slightly more prominent.††

The ranges of time given above cover optimum periods for various makes of dyes and slight changes in concentration. The proper intervals for the stain on hand should be determined by trial each time it is made up, and care taken thereafter that the acetone does not evaporate. It may also be noted that in certain cases of pathologic blood, a longer period sometimes gives a much better result.

*From the Pathological Laboratory, Agnew State Hospital, Agnew, Calif.
Received for publication, Sept. 25, 1925.

**The methylene azur was prepared by oxidation of methylene blue with sodium peroxide.

†Cover the smear with the stain, and allow the adhering solution to remain at least 30 seconds. Wash with tap water. The staining effect is due to evaporation of the acetone. If the stain is not shaken from the slide, it must be allowed to stand several minutes and then diluted as with the Wright method. Care should be taken that the acetone does not evaporate from the staining solution.

††As a substitute for the above offered staining solution we have lately used the "Soloid" tablets. The Romanowsky stain (Wright's modification) gave the most satisfactory results.

Two tablets are ground in a mortar and 10 c.c. of 75 per cent acetone added, the solution is filtered and ready for use. The smear is covered with a few drops of the stain, the excess stain is shaken off, and the smear allowed to stand from 2 to 3 minutes, then washed with tap water.

The resulting picture compares very favorably with Wright's or any of the other conventional blood stains, and is very similar to them in its effect. The granules, and particularly the neutrophilic granules, are brought out very distinctly. This is especially noticed in some pathologic blood smears, as in leucemia. The red cells show a pale green color* in thin smears, dark green in heavy smears.

Additional advantages of this stain are its rapidity, ease of preparation, its economy, and the fact that no buffer is necessary for dilution or washing.

A SIMPLIFIED MEDIUM FOR THE CULTIVATION OF ENDAMEBA DYSENTERIAE†

By Charles A. Kofoid, Ph.D., Sc.D., and Edna Hannibal Wagener, A.B., A.M.
Berkeley, Calif.

BOECK and Drbohlav[1] have recently described a method for the cultivation of *Endameba dysenteriae* on a medium composed of a coagulated egg slant covered with 10 c.c. of Locke albumen, prepared by shaking the whites of two eggs in 1000 c.c. of Locke's solution and passing the whole through a Berkefeld filter in order to sterilize it. Another medium is made by covering the egg slant with 1 part of human serum and 8 parts of Locke's solution.

In carrying on a series of experiments on the growth of *Endameba dysenteriae* in different kinds of media we have found that either rabbit, rat, cat, or guinea pig blood may be substituted for human serum. The most satisfactory medium was found to be the coagulated egg slant covered with 10 c.c. Locke's solution, containing 0.5 per cent of defibrinated rabbit blood.

This medium is less favorable to the bacteria, which are found with the ameba cultures and quickly lower the P_H, than either Boeck's egg-Locke-serum medium or his egg-Locke-albumen medium. It is also simple to prepare, as fresh, sterile rabbit blood can be easily obtained.

On our rabbit blood medium the endameba multiply rapidly and are frequently found ingesting rabbit red blood cells. It is necessary to transplant the cultures every forty-eight hours in order to keep them going continuously.

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*Sometimes light brown, depending upon the concentration.

†From the Zoological Laboratory, University of California, Berkeley, California.
Received for publication, Oct. 24, 1925.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

Stander, H. J., Duncan, E. E., and Sisson, W. E: Chemical Studies of the Toxemias of Pregnancy. Bull. Johns Hopkins Hosp., June, 1925, xxxvi, No. 6, p. 411.

The elaboration of a completely satisfactory method for differentiating between the various types of late toxemias of pregnancy and the discovery of the etiology of eclampsia are among the leading unsolved problems of the field of obstetrics.

The authors review the blood chemistry studies which have been made in these fields and report their own studies of the blood and urine of normal nonpregnant, normal pregnant, and abnormal pregnant women.

The pregnant women were all near term. In eclampsia cases an endeavor was made to secure specimens immediately after a convulsion.

The technic was as follows: Lithium citrate was used as an anticoagulant.

Nonprotein nitrogen: Folin-Wu.

Urea nitrogen: Van Slyke and Cullen.

Chlorides: Whitehorn.

CO_2: Van Slyke.

K, Mg., and P: Modification of Briggs for colorimetric estimation.

Na: Kramer and Tisdale, and Kramer and Gittelman.

Ca: Briggs modification of Kramer's method.

Iron: Wong.

Results: In normal pregnancy, except in the CO_2 combining power, the changes were very slight. In general there was a tendency toward lower values in NPN and urea N and a decrease in the CO_2 combining power.

There is a very slight tendency toward a lowering of the calcium in normal pregnancy, so slight as to be negligible.

Differentiation between nephritic toxemia and eclampsia was not possible by the blood chemistry determinations.

The authors use the formula $\frac{\text{B.U.N.}}{\text{N.P.N.}}$ to express the ratio of the urea nitrogen as a percentage of the nonprotein nitrogen and the formula $\frac{\text{B.U.N.}}{\text{U. N. \%}}$ to express the urea nitrogen as a percentage of the urea nitrogen in the urine.

In preeclamptic toxemia the ammonia nitrogen of the urine and the blood uric acid were slightly elevated and both ratios were lower than normal. The ratios indicated above, therefore, are of some value in differentiating between the nephritic and preeclamptic toxemias.

The most marked variations from normal were found in eclampsia. Ammonia nitrogen in the urine and the blood uric acid showed definite variations, and the CO_2 level was very low in most cases when coma intervened. The most significant changes were in the blood sugar (increased) and the inorganic P values (3.5 as compared to 2.7 mg. normal).

$\frac{\text{(P)}}{\text{(Ca)}}$ was increased: 32.36 (normal 27.83).

The hyperglycemia is assumed to be due to alterations in the liver cells.

The results are thus summarized:

1. Normal pregnancy: NPN and urea N increased; CO_2 decreased; inorganic elements within normal limits.

2. Nephritic toxemia: Increase of both the ratios noted above; inorganic elements within normal limits.

3. Preeclamptic toxemias: no outstanding variations except slight decrease in the ratios described.

4. Eclampsia: uric acid increased; CO_2 decreased in proportion to intensity of coma; blood sugar increased; increase in the P-Ca ratio which was due to increase of inorganic phosphorus.

Davidson, E. C., and Allen, C. I.: The Blood Glucose Curve in Head Injuries. Bull. Johns Hopkins Hosp., October, 1925, xxxvii, No. 4, p. 217.

It has long been known that injury to the central nervous system may be a cause of glycosuria, and the fact that glycosuria occurs, in a small percentage of cases, after serious head injuries, led Davidson and Allen to study the sugar tolerance in a series of head injuries as soon after the injury as possible and again during convalescence with a view to determining if such studies might serve as an index of the extent and severity of the damage done to the brain tissue.

Tests were made approximately sixteen hours after food was ingested and within twenty-four to forty-eight hours after the injury. In patients who recovered and in whom glucose utilization was delayed, the tests were repeated from a few days to several months later.

Due precautions were taken to minimize the effects of muscular activity and extraneous nervous influences.

Glucose was administered intravenously, 100 c.c. of a 25 per cent solution, taking ten minutes to complete the injection, and blood sugar determinations were made at 15, 30, 45, 60, 90, and 150 minutes.

No fluid was permitted during the observation period. An effort was made to secure synchronous urine samples. The spinal fluid pressure was taken six to ten hours after the test.

Studies were made of twelve concussion cases. The average fasting blood sugar was 82.6 mg., approximately normal. After the ingestion of the sugar, the curve showed a much more gradual fall than normal and the values remained above the fasting level after 90 minutes.

There was no constant relation between the blood sugar disturbance and the spinal fluid pressure.

In eighteen cases of fractured skull the findings were similar but more marked.

The data presented indicate that in serious head injuries there is a profound though temporary derangement of the carbohydrate metabolism. Observations during convalescence showed no significant variations from the normal.

The procedure does not promise much in the way of differential diagnosis. The possible mechanism is discussed.

Kubie, L. S., and Shults, G. M.: Studies on the Relationship of the Chemical Constituents of Blood and Cerebrospinal Fluid. Jour. Exper. Med., Oct. 1, 1925, xlii, No. 4, p. 565.

The paper is divided into two parts, the first concerned with studies made upon patients; the second reporting a continuation of the studies upon dogs.

In the human beings blood and spinal fluid specimens were simultaneously collected after a twelve hour fast and chemical analyses made for reducing substances (Folin and Wu), for chlorides (Whitehorn), and in some cases, for nonprotein nitrogenous constituents (Folin).

Thirty cases representing various conditions in which spinal punctures were done as diagnostic or therapeutic measures were thus studied.

The ratio of spinal fluid sugar to blood sugar varied from 0.33 to 0.81, only those fluids which contained large amounts of blood falling below 0.53. There was no difference between normal and pathologic fluids. There were no cases of acute or tuberculous meningitis or of encephalitis in the series.

No dependence of spinal fluid upon blood sugar was demonstrated, probably because the blood sugar undergoes rapid fluctuations while the spinal fluid sugar is formed and absorbed slowly.

There was no correlation between the spinal fluid sugar and the diseases encountered.

The spinal fluid chloride content is normally higher than the blood chloride content and the determinations seemed to indicate that the higher the blood chlorides, the lower is the ratio between the blood and the spinal fluid. No explanation of this is apparent.

Except in one case, the nonprotein nitrogen was lower in the spinal fluid than in the blood. This case was one of cerebral hemorrhage, and it is suggested that the concomitant low sugar is due to the action of enzymes which arose from inflammatory cellular reaction and which, through proteolysis, may also have been responsible for the nonprotein nitrogen content.

The ratio of spinal fluid to blood was from 0.51 to 0.85 for whole blood and 0.65 to 0.92 for plasma. The fluid level does not depend upon the blood level.

In the animal experiments the findings, in a general way, were comparable.

The general conclusions were that, if due allowance is made for the slow elaboration of spinal fluid and the time required for newformed fluid to emerge from a needle in the cisterna, the amounts of sugar, chlorides, and nonprotein nitrogen in the fluid in a general way follow their concentration in the blood serum. It was evident, however, that for any substance, other factors than its concentration play a part in its distribution between the blood and spinal fluid.

Rich, A. R., and Rienhoff, Jr., W. F.: Bile-Pigment Content of the Splenic Vein. Bull. Johns Hopkins Hosp., June, 1925, xxxvi, No. 6, p. 431.

Determinations were made on specimens obtained at operation and at autopsy using the van den Bergh indirect method.

In four of ten cases the splenic vein blood contained more bilirubin than blood from the splenic artery and peripheral veins, thus evidencing the formation of bilirubin in the spleen.

In pernicious anemia and secondary anemia the bilirubin content of the splenic vein may or may not be higher.

In a case of hemolytic jaundice a preoperative high bilirubin content fell to normal within four hours after splenectomy.

McCordock, H. A.: A Bacillus of the Paratyphoid Group. Bull. Johns Hopkins Hosp., December, 1925, xxxvii, No. 6, p. 412.

A cultural and biologic study of a new organism is reported in an article illustrated with microphotographs. The bacillus was isolated from the spontaneous infectious otitis seen in albino rats and the name Salmonella muriotitis is proposed.

It closely resembles the organisms of the paratyphoid group from which it is differentiated by its inability to ferment rhamnose within twenty-four hours and the absence or very slight production of gas in all carbohydrates except xylose.

Smetana, H.: Experimental Study of Amyloid Formation. Bull. Johns Hopkins Hosp., December, 1925, xxxvii, No. 6, p. 383.

The daily intramuscular injection of 0.4 to 0.6 c.c. of a sterile 5 per cent solution of nutrose produces amyloid in white mice and renders the experimental study of this substance possible.

Amyloid appears in all the parenchymatous organs. The author considers the method of Bennhold the best for the demonstration of amyloid in sections: Apply 1 per cent congo red stain with heat, wash, place in saturated lithium carbonate solution for twenty seconds, and differentiate in 80 per cent alcohol.

This can be applied to paraffin sections which can be cleared. Hematoxylin may be used for nuclei.

Zenker fixation is better than formalin, and the best results follow a twenty-four-hour fixation in cold saturated sublimate solution.

Severe damage to the cells of the parenchyma, infiltration of the cells in the periportal regions and in the capillaries of the liver, increase in the cells of the malpighian bodies of

the spleen and cell infiltration about them are constantly seen before the appearance of amyloid and during its production.

In the areas where amyloid appears there is destruction of connective tissue and elastic fibrils, swelling of the cells of the vessel walls, and dissolution of cell nuclei and fusion into a formless mass in which the first traces of amyloid appear.

Bacterial cooperation in the formation of amyloid is not necessary. The article is illustrated with colored plates.

Starobinsky, A.: The Colloidal Benzoin Reaction Applied to Syphilitic Serum. Presse méd., Paris, 1925, xxxiii, 334.

The antigen extract is prepared as follows:

Fresh horse heart muscle is finely ground, spread in glass plates and dried, first at room temperature and then in the incubator at 37° C. for sufficient time to evaporate completely all moisture. The dried mass is then finely powdered. To one part of this powder 5 parts of ether are added, and the mass extracted for forty-eight hours. It is then filtered, and the heart powder is dried in the incubator until there is no odor of ether.

To the dried mass absolute alcohol is added in the proportion of 1:9, and the powder is extracted in the incubator for 3 days. The filtrate constitutes the stock antigen extract.

To 10 c.c. of this stock extract 15 c.c. of absolute alcohol and 4 c.c. of a 10 per cent solution of benzoin resin are added. The resultant mixture should be clear and transparent.

For the test this solution is further diluted: to 1 c.c. of the antigen extract 10 c.c. of 3 per cent sodium chloride solution is added, the tube shaken, and heated to 45° C.

The test is performed by adding 1 c.c. of this final antigen dilution to 0.4 c.c. of serum inactivated for thirty minutes at 56° C.

Readings are made of the degree of flocculation at twenty-four and forty-eight hours. In the author's studies there was an agreement of 93.7 per cent between this flocculation reaction and the Wassermann.

Jordan, E. O.: The Interconvertibility of "Rough" and "Smooth" Bacterial Types. Jour. Am. Med. Assn., Jan. 16, 1926, lxxxvi, 177.

In a number of bacterial groups two types of cells occur in stock cultures, which produce, on agar plates, two types of colonies: "rough" (*R*), and "smooth" (*S*). Under ordinary conditions *S* and *R* colonies breed true. The two types are correlated with other characteristics: *S* cells form stable suspensions; *R* tend toward spontaneous agglutination. The *S* type is more virulent; acid agglutination optimum is higher for *S* than *R*, and there are serologic differences.

The *R* type predominates in old stock cultures which have been allowed to become partially dry.

Various observers have regarded *S* types as the normal, and *R* as degenerative forms. De Kruif regards $S \rightarrow R$ transformations as a mutation.

Jordan studied 100 stock cultures of paratyphoid bacilli and demonstrated by single-cell isolations (Barber method) that either type of colony may be produced at will from a single cell.

Frequent transfers in veal infusion broth led to the *S* type, while keeping the culture in the same medium without transfer for some time led the production of *R* types. All the coincident characteristics noted above were likewise converted.

Regan, J. C. and Tolstoouhov, A. V.: Characteristic Changes in the Blood in Whooping Cough. Jour. Am. Med. Assn., Jan. 16, 1926, lxxxvi, 181.

Two hundred blood chemical analyses were made in cases of whooping cough to determine if any changes of significance occurred in the blood chemistry.

The PH (89 determinations by electrometric method) was definitely changed, ranging from 7.05 to 7.25, and values as low as 7.01 to 6.98 were occasionally encountered.

The authors feel that there is a decided relationship between the stage and severity of the disease and the PH of the blood serum, the latter being lowered in the severe cases and in the paroxysmal stage.

The phosphorus content (138 estimations by Briggs' method), was decidedly lowered in the plasma, being 2 mg. per 100 c.c. as compared to the normal average of 4.5 to 7.1 mg. in children.

The calcium content (46 determinations by Clark's method), was within normal values.

The average CO_2 content was 50 per cent by volume in 57 determinations.

Novak, E.: Report on Antistreptococcus Serum. Jour. Am. Med. Assn., Jan. 16, 1926, lxxxvi, 189.

Novak epitomizes the replies to a questionnaire sent to twelve surgeons and thirteen obstetricians and gynecologists relative to the therapeutic value of antistreptococcus serum.

In the collected experience of these observers the serum was of little, if any, therapeutic value.

Walters, W., Kilgore, A. M., and Bollman, J. L.: Changes in the Blood Resulting from Duodenal Fistula. Jour. Am. Med. Assn., Jan. 16, 1926, lxxxvi, 186.

No satisfactory explanation has been advanced for the cause of death in duodenal fistula. A clinical and experimental study of the blood chemistry was conducted upon two patients with acute postoperative duodenal fistula and upon sixty dogs in which the condition had been experimentally produced.

An increasing alkalosis and a progressive drop in the blood chlorides was noted, together with a progressive rise in the blood urea, findings which are quite similar to those seen in high intestinal stasis.

The disturbance of the fluid balance seems an important factor, together with the coincident renal dysfunction.

The alkalosis is apparently influenced by the large amounts of acid lost in the duodenal and gastric contents, the latter also appreciably affecting the decrease in the blood chlorides.

These results indicate the advisability of intravenous injections of one per cent sodium chloride solution as a therapeutic measure, which seems to prolong life but is merely palliative in effect. Where the fistula is large or the condition acute, operation is imperative.

Plass, E. D., and Tompkins, E. H.: Placental Transmission, II. Phosphoric Acid Compounds in Fetal and Maternal Serum. Jour. Biol. Chem., June, 1923, lvi, No. 2, p. 309.

Continuing a study of fetal metabolism, the phosphoric acid compounds were determined in fetal and maternal serum by the methods of Bloor.

Eleven cases were studied. Lecithin was definitely increased in the maternal serum at the time of delivery and decreased in the fetal serum.

Inorganic phosphoric acid is diminished in the maternal serum, probably as a result of physiologic hydremia or hydroplasma, while the fetal serum contains quantities comparable to the high values seen in young infants and assumed to represent the optimum for bone construction.

Because of the high lecithin content, the total phosphoric acid content is higher in the maternal than in the fetal serum.

Organic phosphorus compounds lead to somewhat higher values in the fetus than in the mother.

Bogert, L. J., and Plass, E. D.: Placental Transmission, I. The Calcium and Magnesium Content of Fetal and Maternal Blood Serum. Jour. Biol. Chem., June, 1923, lvi, No. 2, p. 297.

The experimental determinations reported were part of a study of placental interchange, determinations being made on specimens of umbilical cord blood and maternal vein blood simultaneously secured.

Determinations were made upon the serum: calcium by the method of Kramer and Tisdale, magnesium by a combination of Kramer and Tisdale's and Briggs' methods.

A striking difference was found in the calcium content which was substantially higher in the fetal serum in the 23 cases studied. This difference cannot be explained wholly by a difference in the dilution in the fetal and maternal circulations. The mechanism is, at present, unknown.

The average values for Ca were 9.1 mg. per 100 c.c. in the maternal serum and 10.9 mg. per 100 c.c. in the fetal serum. The average maternal serum calcium is distinctly lower than the average value for normal women; that of the child at birth usually exceeds the normal range for adults.

There is no variation in the magnesium content though there seems to be some tendency for this to be lower than normal in both the fetal and maternal blood.

Josephs, H.: Postanesthetic Hypoglycemia. A Study of the Etiology of Recurrent Vomiting. Bull. Johns Hopkins Hosp., December, 1925, xxxvii, No. 6, p. 376.

A series of tests made upon children who had been subjected to anesthesia (tonsillectomy) are reported. Following operation from 5 to 6 specimens of urine were collected in the first twenty-four hours and examined for acetone bodies, which were determined by the method of Van Slyke and reported as milligrams of Beta-hydroxy-butyric acid.

Blood-sugar determinations were made by the Fitz modification of Bang's method and a gas analysis of expired air by the Haldane technic.

A definite hypoglycemia was shown to occur within eighteen to twenty-four hours after anesthesia, the degree being apparently dependent upon some function of the child called into play by the anesthesia, rather than upon the anesthetic.

The excretion of acetone bodies is greater than that seen after simple starvation. There seems to be some relation between the rise in blood sugar and the rise in the respiratory quotient.

The relation of the above findings to the occurrence of postanesthetic acidosis is discussed.

Summing up, hypoglycemia of moderate degree occurs with great regularity eighteen to twenty-four hours after anesthesia, usually preceded by an increased respiratory quotient and by an increased excretion of acetone bodies in the urine.

Pucher, G. W.: Quantitative Distribution of Veronal in the Organs in a Case of Veronal Poisoning. Bull. Buffalo Gen. Hosp., December. 1925, iii, 3, 70.

The following method was found applicable and accurate for the quantitative isolation of veronal.

I. *For Urine.*—One hundred c.c. of urine are treated with a 20 per cent soution of lead acetate ($2H_2O$) until a precipitate no longer forms (about 15 to 20 c.c.), and the suspended precipitate is brought down by heating on a water-bath. It is then filtered by suction and washed twice with 10 to 20 c.c. of water, is then sucked dry, and the filtrate is transferred to an evaporating dish and concentrated on a steam bath to a volume of 25 to 30 c.c. It is cooled, acidified with 5 per cent sulphuric acid and filtered by suction if necessary. The aqueous solution is extracted twice with 20 c.c. of petroleum ether. The petroleum ether is discarded and the solution is extracted three times with 15 to 20 c.c. of redistilled ethyl acetate (b.p. 77-79° C.).

The ethyl acetate extract in a small Erlenmeyer flask (100 c.c.) is shaken for about five minutes with 5 to 6 grams of anhydrous sodium sulphate and is then filtered through a dry filter paper into a 100 c.c. beaker. Residual sodium sulphate is washed by decantation three times with 5 c.c. of ethyl acetate and the solid is then transferred to the filter paper with a few cubic centimeters of the solvent and allowed to drain thoroughly. It is evaporated to dryness on a water-bath, and the residue taken up by warming with 10 c.c. of water and 5 c.c. of 5 per cent sulphuric acid. It is cooled and shaken for one minute with one gram of Lloyd's reagent (purified Fuller's earth), and is filtered by suction, the precipitate being washed twice with 5 c.c. of water. The colorless filtrate is now heated to boiling and 5 per cent potassium permanganate added drop by drop until a faint pink color persists. It is filtered by hot suction. The filtrate is decolorized with a few drops of 3 per cent hydrogen

peroxide and extracted three times with 15 to 20 c.c. of ethyl acetate. The extract is again heated with 5 to 6 grams of anhydrous sodium sulphate and filtered through a dry paper into a weighted 100 c.c. beaker containing a few pebbles. It is evaporated to dryness on a steam bath, and then dried to constant weight at 60 to 70° C. The residue so obtained is a white crystalline solid which after weighing can be identified in the usual way.

II. *Organs.*—Weighed portions of the organs (100 to 500 grams) are minced as finely as possible and transferred to an appropriate Erlenmeyer flask containing 200 c.c. of 95 per cent ethyl alcohol and 30 c.c. of 5 per cent sulphuric acid. They are immersed in a steam bath and boiled with frequent shaking for forty-five to sixty minutes; then filtered hot onto a suction funnel, the juices being pressed out from the residue as completely as possible. The residue is transferred to a mortar and ground with 200 c.c. of ethyl alcohol, and again transferred to the Erlenmeyer flask and boiled for thirty to forty minutes. It is filtered hot, as previously described. The extraction is repeated at least twice or more, depending upon the amounts and fineness of division of the tissues. The alcoholic extracts are combined and, after cooling, are filtered by suction. They are concentrated on a steam bath until all the alcohol has been removed. The solution should be made up to about 150 c.c. with distilled water, 20 per cent lead acetate being added until no further precipitation occurs, and then treated with procedure followed in *Urine*.

With the above procedure 98 to 100 per cent recoveries of veronal from urine, and 92 to 98 per cent recoveries from organs, were obtained.

Most of the veronal is found, as a rule, in the urine, the kidney and brain containing the next largest amounts.

University of Bordeaux—A Special Course

A special course of five weeks' duration, beginning July 8, 1926, will be given by Professor Georges Portmann at the University of Bordeaux, Post Graduate School of Oto-Rhino-Laryngological Department. This course is pronounced as the best course on the continent.

Gross and minute anatomy and physiology of the ear, nose, throat, larynx and neck will be taken up in detail. Instruction will also be given in bronchoscopy, esophagoscopy and direct laryngoscopy, as well as in special treatments in oto-rhino-laryngology.

The class will be limited to twelve students and enrollment will close June 1, 1926. Fee for the course is $200.00.

For further information, apply to Dr. Leon Felderman, 4428 York Road, Philadelphia, Pa.

BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.)

*The Diabetic Diet**

AMONG the diabetic manuals this will find a definite place. That it was developed in Toronto and has the endorsement of Banting and Gilchrist should be sufficient recommendation.

There are on the market several manuals for the use of diabetic patients, varying from the rather detailed work of Joslin, in the reading of which the patient becomes thoroughly acquainted with his condition and must learn to calculate his diet with exactitude, down to those in which just sufficient information is given to enable the average diabetic to understand the basic principles of his disease and its treatment, and in which cut and dried diets are incorporated. While the authors describe them as sample menus to be altered for the individual case, we will venture to say that most patients and a large proportion of physicians base their entire treatment upon these unaltered groups of sample menus.

The reviewer feels that the most useful diabetic manual will be one with sufficient "sample menus" to cover the usual range of necessary diets, and in which provision is made for easy substitution without vitiation of the food values. In other words, skeleton diets should be provided, onto which may be built other foods in the proper proportion, selected from simple food tables.

This manual provides skeleton diets, and provides tables for the variation of vegetables and fruits. Variation of other foods such as cereals, meats, etc., is not easy. We should like to see a greater number of skeleton diets. Perhaps the best dietary schedule so far proposed for general use is that found in Mosenthal's article in Tice's "Practice." The volume under review is as practicable as any of the diabetic manuals available, however, and better than some.

Methods in Surgery†

THIS appears as a companion piece to Herrmann's *Methods in Medicine.* Both of them deal with the hospital and staff routine of the Barnes Hospital and other institutions in St. Louis, Mo. The book may be roughly divided into three sections. The first and largest contains tabloid directions for general and special surgical examinations, hospital directions for preoperative and postoperative care, and hospital rules and regulations. The second por-

*Diabetic Diet, a handbook for diabetics. By A. Doris McHenry, B.A., and Marjorie M. Cooper, B.A. with preface by J. A. Gilchrist and F. G. Banting. Cloth. Pp. 70. Harper & Brothers, New York City. 1925.

†Methods in Surgery. By Glover H. Copher, M.D., Instructor in Surgery, Washington University School of Medicine, Clinical Assistant to Barnes Hospital, Surgeon to out-patient, Washington University Dispensary, visiting surgeon, St. Louis City Hospital. Cloth. Pp. 232. Price $3.00. C. V. Mosby Co., St. Louis, 1925.

tion, contributed by Marjorie H. Copher, contains routine and special diets used on the surgical service. The last portion, which is in the nature of an appendix, presents all the special charts used on the surgical service.

The volume will be of particular interest to any who wish to work up efficient and satisfactory hospital routines and regulations.

*The Normal Diet**

A SMALL brochure containing in very readable form the meat of a series of lectures given by the author to certain groups of patients, in which he stresses the normal metabolic requirements of the body. He presents the subject in such a manner as to be easily comprehended by the average layman, so that the latter may carry away with him an accurate knowledge of how to apply the principles of human nutrition.

Empyema Thoracis†

THIS monograph is the Samuel D. Gross prize essay accepted by the Philadelphia Academy of Surgery. In it the author discusses the newer principles of the surgical treatment of empyema that have developed since our experience with the disease during the World War. The cardinal principles emphasized by the author are: (1) careful avoidance of open pneumothorax in the acute stage; (2) the prevention of a chronic empyema by the rapid sterilization and obliteration of the infected cavity; and (3) careful attention to the nutrition of the patient. He deals with general principles rather than details and incorporates extensive discussions of the pathology, and of results with experimental empyema and pneumonia. He prefers repeated aspirations during the acute stage to continual drainage with negative pressure and recommends the Carrel-Dakin solution for sterilization and obliteration of the cavity. In a rather long addendum he surveys all criticisms of his principles, which have been made since the first presentation of his paper.

This monograph, together with Vol. II, part 2, of the report of the Medical Department of the U. S. A. in the World War, forms a very complete reference library on surgical treatment of empyema.

The Nursing of Eye Cases‡

A SMALL brochure for the use of nurses, describing the anatomy of the eye, particularly the external anatomy, and its appearance in the various common eye diseases and injuries. The author does not stress so much what is to be done in the individual case as how it should be done after the physician has so ordered.

*The Normal Diet. By W. D. Sansum, M.S., M.D., Director of the Potter Metabolic Clinic, Dept. of Metabolism, Santa Barbara, Calif. Cloth. Illustrated. Pp. 72. Price $1.50. The C. V. Mosby Co., St. Louis, Mo., 1925.

†Some Fundamental Considerations in the Treatment of Empyema Thoracis. By Evarts A. Graham, A.B., M.D. Cloth. Illustrated. Pp. 110. Price $2.50. The C. V. Mosby Co., St. Louis, Mo. 1925.

‡By Louise Kingham, S.R.N., Matron of the Weymouth and Dorset County Royal Eye Infirmary. Paper. Pp. 16. Price thirty cents. Humphrey Milford. Oxford Univ. Press.

*Abdominal and Pelvic Surgery for Practitioners**

THIS is a treatise on the diagnosis of abdominal and pelvic diseases, both acute and chronic, in which the author presents systematically those guiding principles of diagnostic generalizations which have served him to excellent purpose during his career as a surgeon and which he illustrates with case reports from what appears to have been a wealth of clinical material.

The author divides the work into three sections, abdominal emergencies, subacute abdominal diseases, and chronic abdominal diseases.

While most attention is devoted to the diagnosis of abdominal emergencies, conditions requiring immediate operation, the essential conservatism of the author is brought out at many points in the book. We feel that this adds weight to his opinions. Thus he designates when it is best not to operate in general peritonitis, even from a perforated ulcer, the patient's best chance for recovery being by avoidance of operation. He describes the large number of operations that are performed for so-called chronic appendicitis. While there is such a condition, true chronic appendicitis is almost always associated with an antecedent acute attack.

Personal and Community Health†

INCREASING interest in individual preventive medicine necessitates the development of textbooks and monographs on this subject in which is collected the literature which is now scattered through many and diverse volumes. Dr. Turner has done this very successfully. The keynote of the work is personal health and hygiene. Of necessity community health comes in for discussion together with general principles of preventive medicine, but the chief reasons for their presentation is to outline practical measures for the maintaining of personal health. Thus food control, water supply, waste disposal, public health administration, school hygiene, industrial hygiene, ventilation, heating and lighting, are as important to the health of the individual as are proper nutrition, exercise, reproduction, oral hygiene, and mental hygiene.

The first appendix contains an alphabetical list of infectious diseases with brief descriptions of the infectious agent, source of infection, mode of transmission, incubation period, and period of communicability, followed by an outline of methods of control. The second appendix discusses disinfection and the various disinfectants.

The book should find a wide market.

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*Abdominal and Pelvic Surgery for Practitioners. By Rutherford Morison, Hon.M.A. and D.C.L., Hon. LL.D., M.B., F.R.C.S., (Ed. and Eng.). Cloth. Pp. 212. Humphrey Milford. Oxford Univ. Press. 1925.

†Personal and Community Health. By Clair Elsmere Turner, Associate Professor of Biology and Public Health in the Mass. Institute of Technology; Associate Professor of Hygiene in the Tufts College Medical and Dental Schools; etc. Cloth. Illustrated. Pp. 426. Price $2.50. The C. V. Mosby Company, St. Louis, Mo. 1925.

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EDITORIALS

The Choice of a Pathologist

IT is very obvious to the conscientious practitioner of medicine that the day of the man who knows it all is past, and while, to a certain type of mind, there may be some satisfaction in the assumption of omniscience for the benefit of the gaping multitude, some pabulum for the vanity in the thought that "still the wonder grew that one small head should carry all he knew," the reputation so founded is built upon sand and but the wonder of an hour.

The scientific practice of medicine revolves around the formation of a diagnosis. The formation of a diagnosis, with the subsequent elaboration of an intelligent plan of treatment, evolves from the collection, analysis, and correlation of minutia, of data procured by various means and from divers sources.

It is essential, therefore, for the physician to be familiar with the means, methods, and sources for acquiring this necessary information and capable of utilizing and applying the interpreted results to the problem at hand.

It is apparent to the most casual observer that the busier the practitioner, the more often he will require a varied investigation of his cases; and the less time, to mention only one requirement, will he have available to collect at first hand the information sought. The more necessary will it become, then, for him to utilize the specialized services of skilled associates.

We may expect of him that he shall suspect the existence of a neurologic condition or the presence of a malignant neoplasm, but we cannot demand of him in all contingencies the specialized training necessary to render a possible diagnosis absolute.

It may, however, be demanded that he shall take advantage of all means available to establish or rule out a diagnostic possibility, as, for example, by consultation with a colleague of particular skill in such matters, or through utilization of the various avenues of laboratory investigation of significance, pro or con.

It may even be said, all things being equal, that one index of the thoroughness with which a diagnostic problem is studied is the degree to which the resources of consultation are utilized, the care with which every possibility is investigated.

To seek corroboration or elimination of a possible diagnosis is not a confession of ignorance but a manifestation of wisdom; to pretend knowledge in the face of doubt or to neglect a thorough study is little less than criminal.

Considering the care with which consultants are, or should be, chosen; the value justly given to skill, training, past experience, and general reputation, one is sometimes at a loss to explain the carelessness attendant upon the selection of a clinical pathologist by the average general practitioner.

He who selects his surgeon, his radiographer, with meticulous care chooses his laboratory consultant haphazard.

Perhaps this is due, in no small measure, to an ill-advised confusion of the laboratory and the pathologist, to the habit of looking upon the two as synonymous and interchangeable terms, whereas nothing could be further from the truth.

The scalpel and the surgeon or the radiographer and his apparatus are not transposable, though one is complemental to the other, and the same is true of the clinical pathologist.

The clinician should demand of the pathologist not only the training and skill required to insure accurate and reliable findings, but, in case of need, the ability based upon experience, reading, medical education and clinical training necessary to render him available as a consultant, either from the standpoint of indicating the plan of laboratory approach most likely to be informative, or of assisting in the clinical evaluation of the results obtained.

Unfortunately for the practitioner who carelessly casts his laboratory work to the winds; who chooses because it is labeled "laboratory"; because it is near by, pretentious in appearance, or persistent in self-adulation, the necessity for extensive use of laboratory methods has resulted, by what Webster has called "the fearful concatenation of circumstance," in a mushroom growth of "laboratories" not always indicative of nor accompanied by

the coincident presence of a clinical pathologist, by which term is denoted a physician of clinical training and experience devoting himself to pathology as a specialty.

All too often the "laboratories" clamoring for the physician's "business" are entirely lay owned and lay manned, seeking by an obtrusive display of glittering apparatus and the clamorous use of technical patter to "varnish nonsense with the charm of sound."

To him who, unthinking, holds the test to be the thing; who believes that technicians can be trained in a few weeks to cover the entire range of laboratory procedures, the words of Oliver Wendell Holmes may be recalled, "Knowledge, like timber, should not be much used until it is seasoned."

The practice of clinical pathology is the practice of specialized medicine, and the technician can no more replace the clinical pathologist than the nurse or the first year student can supplant the physician.

The training of technicians is restricted entirely to methods, and, just as the clinician places the responsibility for his operations only on the surgeon, so should he place the responsibility for his laboratory studies only on the clinical pathologist. And just as he chooses his surgeon on the sole basis of skill and experience, so should he demand equivalent qualifications from his laboratory consultant.

He should appreciate, also, that to the layman the laboratory is merely a business, while to the clinical pathologist it is a profession of equal dignity and learning and subject to the same ethical and professional responsibilities as any other branch of medicine.

The man who calls the laboratory on the phone to discuss a report or talk over a case should realize that he is in consultation with a professional colleague and should demand that this be the case and not find himself debating professional problems with law technicians who may later, perhaps, be "consulting" with a cultist or even a quack.

The practitioner should always be in a position to know who does his laboratory work and be fully cognizant of his training and ability.

Less than this is an injustice to the patient and an indictment of the physician. —*R. A. K.*

The Need for a Fixed Hemoglobin Standard

A HEMOGLOBIN estimation, properly made, is one of the most valuable clinical laboratory procedures. Today widely different results may be obtained by the same specimen of blood by different laboratory workers. The clinician is often at a loss to evaluate properly the hemoglobin percentage as reported to him.

Some of the confusion is due to technical errors in apparatus for hemoglobin determination. It is not uncommon to find the standard and graduated tube of a Sahli apparatus of widely different bore. Pipettes are not infrequently incorrectly calibrated. The combined error in such an apparatus checked recently was 40 per cent.

In direct hemoglobin determination, as with the Van Slyke blood gas apparatus,[1] the hemoglobin is measured in grams per 100 c.c. of blood. For clinical application this is best expressed in percentage. In the various colorimetric procedures, the hemoglobin is read off directly in percentage of a fixed standard. In any event it is necessary to know the equivalent of the 100 per cent in terms of grams per 100 c.c. of blood.

It is usually stated that 100 per cent on a hemoglobinometer should represent the average normal number of grams of hemoglobin per 100 c.c. of blood. The hemoglobin content varies with age and sex and red cell count. In calculating the color index we consider five million cells per cubic millimeter as 100 per cent cells. The most logical standard is certainly one which takes as 100 per cent the average value of hemoglobin in grams per 100 c.c. for each five million red cells.

There are wide differences in normal hemoglobin values as given by different authors and workers. Leichenstern's[2] figures for work done in 1878 are quoted in some textbooks. Williamson,[3] using the same spectro-photometric method, obtained much higher results. Haldane's standard[4] of 13.8 grams per 100 c.c. is still used by many. This represents an average of only 12 determinations with the oxygen capacity method. No one of the workers quoted has made simultaneous red cell counts, so the figures cannot be expressed on a basis of five million red cells per c. mm. Most observers agree that the Haldane standard is too low. Newcomer,[5] in his new method, uses Williamson's figures for the normal.

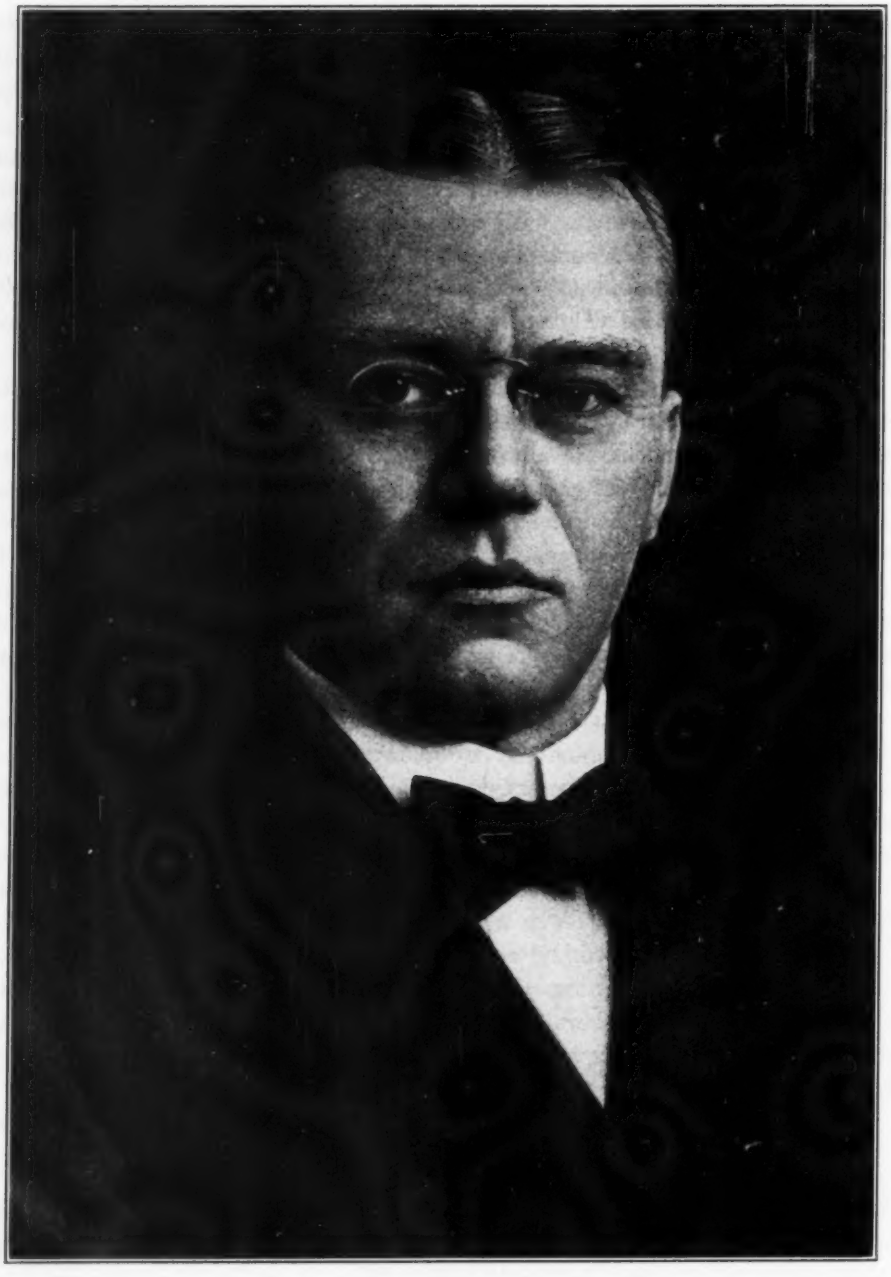
It is obvious that the standard figures used must be based on an accurate method which is available generally to laboratory workers. The oxygen capacity method as adapted by Van Slyke to his blood gas apparatus is certainly the method of choice. The logical standard should take as 100 per cent, the number of grams of hemoglobin per 100 c.c. of blood, in a normal individual having five million cells of normal volume and hemoglobin content per c. mm., as determined by the Van Slyke apparatus. One series of determinations on this basis has been reported.[6]

The American Association of Clinical Pathologists could render a distinct service to laboratory workers by having a committee study the hemoglobin standard question. In this way an authoritative standard could be determined for all laboratory workers to clarify the situation.

REFERENCES

[1]Van Slyke, D. D., and Stadie, W. C.: Jour. Biol. Chem., 1921, xlix, 1.
[2]Leichenstern, quoted by Sahli: Diagnostic Methods, Philadelphia, 1911, W. B. Saunders Co., p. 742.
[3]Williamson, C. S.: Arch. Int. Med., 1916, xviii, 505.
[4]Haldane and Smith: Jour. Physiol., 1899-1900, xxv.
[5]Newcomer, H. S.: Jour. Biol. Chem., 1923, lv, 565.
[6]Haden, R. L.: Jour. Amer. Med. Assn., 1922, lxxix, 1496.

—*R. L. H.*



DR. FREDERIC E. SONDERN
New York City, N. Y.
President, American Society of Clinical Pathologists, 1925-1926

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On to Dallas—to the Fifth Annual Meeting

American Society of Clinical Pathologists

THE Fifth Annual Meeting of the American Society of Clinical Pathologists will be held in Dallas, Texas, April 15, 16, and 17, 1926, at the Baker Hotel.

The members are looking forward to this annual event with a great deal of interest and pleasure, not alone on account of its scientific value but also because of the friendships renewed and new and pleasant acquaintances formed at these reunions. It is a great stimulus to meet clinical pathologists from the larger and smaller medical centers and exchange views. The meeting forms an incentive to better work; it tends to rouse us from apathy and incites us to research and progress.

A number of important problems are to be discussed at the meeting aside from the scientific papers which in themselves offer a variety of demonstrations of interest to our specialty. A proposition that will be discussed relates to the question of standardization of laboratories or, as we prefer to term it, the improvement of the scientific status of the clinical pathologist. Considerable progress has been made in this direction by surveys instituted by our Society and other organizations, the results of which will be presented at the business session of our annual convention. Another subject of paramount importance is the valuable suggestion of Past-President John A. Kolmer of an official text of approved laboratory methods in clinical pathology. In accordance with this proposal committees will be selected from the membership to study the value of various methods and they will report it subsequently as a guide for the editors of this digest.

Questions of economic import will also have a place on the program. The spector of state medicine now looming up strongly before the medical profes-

DR. WILLIAM G. EXTON
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Philadelphia, Pa.
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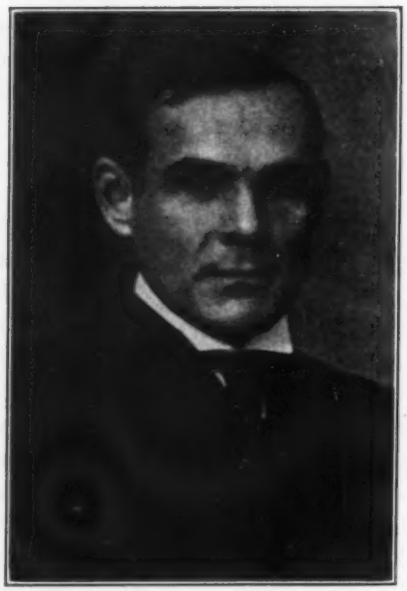

DR. F. L. BURNETT
Boston, Mass.
Vice-President

DR. WARD BURDICK
Denver, Colorado
Secretary-Treasurer

sion and threatening our specialty more than any other is a problem to be reckoned with and which will be given due place in the discussion. The relation of public health laboratories to private practitioners in clinical pathology will be considered by members from the respective groups.

Other questions to be taken up will be the matter of an official publication; the awarding of a prize to stimulate original research and similar matters of interest.

Those who were privileged to be present at the Philadelphia convention will always remember with pleasure the valuable information and the intellectual treat they received during the proceedings. We anticipate a similar if not a greater thrill at the coming Dallas meeting.

All preparations that our Southern members have made presage a wonderful and pleasant gathering. In short a very lively time is anticipated for the next convention.

PROGRAM

American Society of Clinical Pathologists

BAKER HOTEL, DALLAS, TEXAS

April 15, 16, 17, 1926

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Dr. William Carpenter MacCarty, Rochester, Minn., 1923-1924.
Dr. John A. Kolmer, Philadelphia, Pa., 1924-1925.

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St. Louis, Missouri
Member of Board of Censors

DR. ERNST A. VICTORS
San Francisco, Calif.
Member of Board of Censors

A familiar snapshot of President-Elect Wm. G. Exton, and Dr. Herman Spitz, Chairman of the Executive Committee on the Boardwalk at Atlantic City.

THURSDAY, APRIL 15, 9 A.M.

Call to Order—Appointment of Committees

Scientific Program

Hemoglobin and Erythrocytes in the South.

By Dr. Leon S. Lippincott, Vicksburg, Mississippi.

A Combined Diluting and Staining Fluid for Differential Leucocyte Count in the Counting Chamber.

By Dr. Daniel Nicholson, Winnipeg, Canada.

Sickle Cell Anemia.

By Dr. G. S. Graham, Birmingham, Alabama.

A Photographic Method for Counting Blood Cells.

By Dr. A. H. Sanford, Rochester, Minnesota.

The Baker Hotel
Headquarters, American Society of Clinical Pathologists, April 15, 16, and 17, 1926.

THURSDAY, APRIL 15, 2 P.M.

Determination of Sugar in Normal Urine.

By Dr. Mark R. Everett, Norman, Oklahoma.

The Glucose Tolerance Test.

By Dr. W. B. Lewis, Battle Creek, Michigan.

A Study of the Pigment in Addison's Disease.

By Dr. Carl L. Spohr, and Dr. Robert A. Moore, Columbus, Ohio.

Ochronosis.

By Dr. Ernest Scott and Dr. Robert A. Moore, Columbus, Ohio.

The Treatment of One Hundred and Twenty-Five Cases of Acid Intoxication with the Buffer Solutions.

By Dr. F. A. Hecker, Ottumwa, Ia.

Clinical Results with Pathogen.

By Dr. Otto Lowy, Newark, N. J.

FRIDAY, APRIL 16, 9 A.M.

Frozen Sections, Their Place, Value, and Methods.
By Dr. L. A. Turley, Norman, Oklahoma.

Laboratory Examinations Necessary and Unnecessary.
By Dr. George L. Schadt, Springfield, Massachusetts.

The Integration of Hospital Laboratory Work.
By Dr. Philip Hillkowitz, Denver, Co'orado.

The Cytomorphosis of the Tubercle Bacillus.
By Dr. Harry J. Corper, Denver, Colorado.

Oxygentherapy.
By Dr. Paul Roth, Battle Creek, Michigan.

A Key to the Diagnosis of Neoplastic Conditions.
By Dr. William Carpenter MacCarty, Rochester, Minnesota.

FRIDAY, APRIL 16, 2 P.M.

Intestinal Amebiasis from the Pathological Standpoint as Related to the Clinical, with Preliminary Report of X-Ray Studies of Early Cases.
By Dr. J. M. Feder, Panama.

Treponematosis as Seen in the Rural Population of Haiti.
By Dr. C. S. Butler, Port-au-Prince, Haiti.

The Hirsch-Abderhalden Test.
By Dr. F. E. Sondern, New York City.

Comparison of Kolmer and Kahn Tests.
By Dr. C. E. Roderick, Battle Creek, Michigan.

Comparative Result with the Vernes and Wassermann Tests.
By Dr. L. H. Cornwall, New York City.

FRIDAY, APRIL 16, 7 P.M.

Annual Dinner at the Baker Hotel

Presidential Address.
By Dr. Frederic E. Sondern, New York City.

Greetings from the American College of Surgeons.
By Dr. M. T. MacEachern, Chicago, Illinois.

Welcome to Dallas.
By Dr. Edward F. Cooke, Houston, Texas.

SATURDAY, APRIL 17, 9 A.M.

Business Session

What Has Been Done in the Last Year by the American Medical Association in Regard to Standardization of Laboratories.
By Dr. N. P. Colwell, Chicago, Illinois.

SATURDAY, APRIL 17, 2 P.M.

Business Session

Commercial Exhibits

Laboratory workers, more than most any other group of physicians, need to keep in close touch with the manufacturers of the supplies and equipment which they need to carry on their work successfully.

The Executive Committee of the American Society of Clinical Pathologists decided in 1925 that the members and visitors at the annual meeting of the Society would derive a great deal of good from a commercial exhibit by the manufacturers who make and sell the articles used in the medical laboratory.

Such an exhibit was held in conjunction with the Philadelphia Meeting, and it proved to be an outstanding success both for the members and the exhibitors. Such an exhibit will be held at the Dallas Meeting, and members of the Society and their friends attending the meeting will be given an opportunity to meet representatives of the firms from whom they purchase their supplies. Such a meeting is always mutually profitable. Firms which supply laboratories with the essential materials and equipment to carry on successfully are rendering no less a service to medicine and humanity than are the laboratory workers themselves.

There would be no laboratories without instruments of precision, reagents, and stains. If this exhibit succeeds in establishing a closer working relationship between the laboratory and the firms making the supplies and equipment, then everyone connected with it will be content. Dr. L. A. Duck will be in charge of the exhibits.